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Original article

Tobacco

Moist smokeless tobacco (Snus) use and risk of Parkinson's disease

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Abstract

Background: Cigarette smoking is associated with a lower risk of Parkinson's disease. It is unclear what constituent of tobacco smoke may lower the risk. Use of Swedish moist smokeless tobacco (snus) can serve as a model to disentangle what constituent of tobacco smoke may lower the risk. The aim of this study was to determine whether snus use was associated with a lower risk of Parkinson's disease.

Methods: Individual participant data were collected from seven prospective cohort studies, including 348 601 men. We used survival analysis with multivariable Cox regression to estimate study-specific relative risk of Parkinson's disease due to snus use, and random-effects models to pool estimates in a meta-analysis. The primary analyses were restricted to never-smokers to eliminate the potential confounding effect of tobacco smoking.

Results: During a mean follow-up time of 16.1 years, 1199 incident Parkinson's disease cases were identified. Among men who never smoked, ever-snus users had about 60% lower Parkinson's disease risk compared with never-snus users [pooled hazard ratio (HR) 0.41, 95% confidence interval (CI) 0.28-0.61]. The inverse association between snus use and Parkinson's disease risk was more pronounced in current (pooled HR 0.38, 95% CI 0.23-0.63), moderate-heavy amount (pooled HR 0.41, 95% CI 0.19-0.90) and long-term snus users (pooled HR 0.44, 95% CI 0.24-0.83).
Conclusions: Non-smoking men who used snus had a substantially lower risk of Parkinson's disease. Results also indicated an inverse dose-response relationship between snus use and Parkinson's disease risk. Our findings suggest that nicotine or other components of tobacco leaves may influence the development of Parkinson's disease.

Key words: Parkinson's disease, individual participant data, risk factors, epidemiology, nicotine

Key Messages

- Non-smoking men who used snus had a 60% lower risk of Parkinson's disease compared with never snus users.
- An inverse dose-response relationship between snus use and subsequent risk of Parkinson's disease was indicated.
- Nicotine or other components of tobacco leaves may influence the development of Parkinson's disease and explain the inverse relationship between cigarette smoking and risk of Parkinson's disease.
- This is the first pooled analysis of individual participant data from prospective cohort studies that addresses the issue of a potential protective effect of nicotine or other components of tobacco leaves from smokeless tobacco on Parkinson's disease risk.

Introduction

The aetiology of Parkinson's disease remains poorly understood, whereas epidemiological evidence has consistently demonstrated an inverse association between cigarette smoking and risk of Parkinson's disease.[1] Despite the well-known adverse health consequences of smoking, the risk of Parkinson's disease is about 36% lower in ever-smokers compared with non-smokers,[2] and the risk is lower with heavier and longer duration of smoking.[3] The exact mechanism explaining the reduced risk of Parkinson's disease with smoking is unclear, but nicotine may be implicated, as shown to have a neuroprotective effect in parkinsonian animal models.[4] Contrasting the relationship with Parkinson's disease between use of different tobacco products, and between smokeless and smoked tobacco in particular, may shed further light on what constituent of tobacco smoke may be responsible for the reduced Parkinson's disease risk, if causal.

A strong inverse association between smokeless tobacco use, including chewing tobacco or snuff, and Parkinson's disease risk was reported in two small studies hitherto comprising the science base.[5,6] Snus is a Swedish oral moist smokeless tobacco product that delivers a dose of nicotine into blood equivalent to cigarette smoking, but without toxic combustion yields from cigarettes.[7] Snus is used by Swedish men more frequently than cigarettes, with a prevalence at about 18%,[8] and the sales of snus are increasing in the USA.[9,10]

To evaluate the association between snus use and Parkinson's disease risk, we collected individual participant data from the Swedish Collaboration on Health Effects of Snus Use, which brought together Swedish prospective cohort studies with detailed information on tobacco smoking and snus use. Here we present results from this first-ever pooled analysis of the association between snus use and Parkinson's disease risk, based on the largest sample to date.

Methods

Study design, setting and participants

The Swedish Collaboration on Health Effects of Snus Use is a national pooling project, including individual participant data from already established prospective Swedish

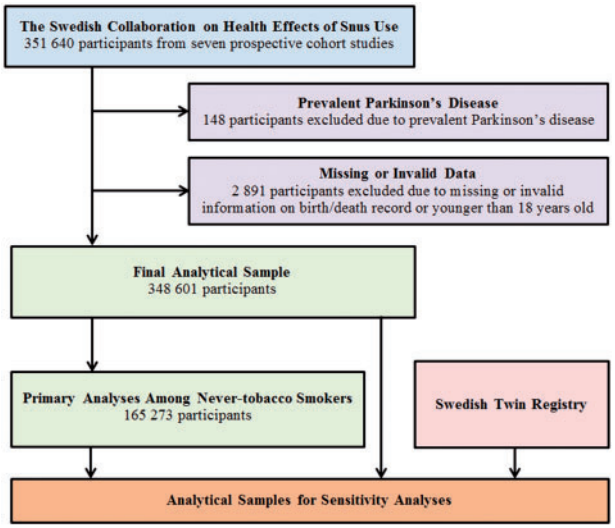

Figure 1. Flowchart deriving the analytical sample.

Additional sensitivity analyses using data from the Swedish Twin Registry were conducted to assess whether potential misclassifications or changes of exposure over time attenuated the association, and whether there was a dose-response relationship of years of lifetime snus use with Parkinson's disease risk (full details are provided in Supplementary Methods).

cohort studies with information on both snus use and tobacco smoking. In total, seven cohorts were included in present study. Participants were excluded if they had a Parkinson's disease diagnosis prior to study enrolment or were younger than 18 years old. The present study was restricted to men, due to low prevalence of snus use in women. Figure 1 illustrates the process of derivation of the analytical sample. Details on study design and measures regarding the collaborative pooling project, as well as the individual studies, have been reported elsewhere.[11–18]

Principal investigators of each cohort study provided raw individual participant data to the collaborative pooling project. Data handling and analyses were performed centrally.

Ascertainment of outcome

Parkinson's disease cases were identified from the Swedish National Patient Register[19] and the Cause of Death Register.[20] The Swedish National Patient Register, which started in 1964 and became nationwide in 1987, includes records on hospital discharge diagnoses as well as outpatient visits since 2001. Diagnoses in the national patient register were coded according to the Swedish Revisions of the *International Classification of Diseases (ICD)*. The Cause of Death Register covers all death records coded in Sweden since 1961. ICD codes used for Parkinson's disease were: 350 (ICD-7, 1964–68), 342 (ICD-8, 1969–86), 332.0 (ICD-9, 1987–96) and G20 (ICD-10, 1997–2013). Index date of Parkinson's disease was defined as the date of first-ever primary or secondary Parkinson's disease diagnoses in the national patient register, or the date of underlying cause of death in the death register. A previous validation study showed a positive predictive value of 70.8% and a sensitivity of 72.7%, comparing hospital discharge diagnoses of Parkinson's disease against clinical diagnoses.[21]

Data collection

Information on tobacco use was collected at baseline using self-reported questionnaires in six studies[12–15,17,18] and by a structured telephone interview in one study.[16] All seven studies contributed information on regular use of snus and tobacco smoking (never, former or current use). Tobacco smoking was classified as lifetime regular use of any type of smoked tobacco products (e.g. cigarettes, pipes and cigars). Information about average amount (cans per week) and duration (years) of snus use for current-snus users at baseline was available in all studies.

Covariates collected at baseline included coffee drinking, alcohol intake, educational level and physical activity level.[1,22] Information on coffee drinking (number of cups per day) was available in two studies (Screening Across the Lifespan Twins, SALT, and Vasterbotten Intervention Programme, VIP),[16,18] and categorized into three groups: 'never', '1 to 3 cups per day' and 'more than 3 cups per day'. Information on alcohol intake was available in all studies except one (Construction Workers Cohort, CWC).[13] Total amount of alcohol intake from all types of alcoholic beverages was calculated (grams per week) and then categorized as 'never', 'low', 'medium' and 'high' intake (in tertiles). Highest achieved level of education, categorized as 'compulsory school', 'secondary or high school' and 'university or above', was available from all studies except the CWC.[13] Information about physical activity level was available in five studies[12,14–17] and categorized into four groups, approximately equivalent to 'less than 2 h light activity per week', 'more than 2 h light activity per week', '1–2 h of exercise per week' and 'more than 2 h exercise per week'. Full details about the covariates and information sources in each cohort are provided in Table 1.

Statistical analysis

Use of snus and smoking were primarily categorized into 'never' and 'ever' use, of which 'ever' use was further categorized into 'current' and 'former' use. For current-snus users, amount was classified as 'light' (less than 2 cans per week) or 'moderate-heavy' (equal to or more than 2 cans per week), except in the National March Cohort (NMC)[15] where 'light' was classified as 'less than or equal to 2 cans

Table 1. Baseline characteristics of participants in seven prospective cohort studies in the Swedish Collaboration of Health Effects of Snus Use (total sample)

Characteristics	CWC	SPHC	MONICA	NMC	SALT	VIP	WOLF
Male participants (*n*)	214 381	39 212	4 553	15 012	18 316	49 940	7187
Period of recruitment (years)	1978–93	2002–10	1986–2004	1997	1998–2003	1992-2013	1992-1997
Age at recruitment (years, mean ± SD)	34.2 ± 12.5	50.2 ± 16.9	49.0 ± 13.4	53.4 ± 17.1	56.6 ± 8.0	47.2±9.2	42.9±10.8
Follow-up time (years, mean ± SD)	20.0 ± 5.1	4.8 ± 3.1	12.5 ± 6.3	12.6 ± 2.5	10.0 ± 1.6	12.4±6.3	13.9±2.1
Age at PD diagnosis (years, mean ± SD)	74.4 ± 9.2	73.9 ± 9.0	73.0 ± 5.7	75.0 ± 8.4	69.4 ± 7.4	66.4±7.3	60.5±9.0
Incident PD cases (*n*)	474	89	31	165	117	291	32
Snus use status (%)							
Never	70.3	70.1	59.8	70.1	74.8	53.6	64.6
Former	3.4	10.2	15.1	14.0	8.5	16.1	11.3
Current	26.4	17.6	23.5	9.1	15.4	26.1	23.0
Missing	0.0	2.1	1.6	6.7	1.4	4.2	1.1
Amount (%)[a]							
Light	30.5	12.3	40.0	51.6	19.4	32.4	15.9
Moderate-heavy	69.5	86.9	60.0	48.3	80.1	67.6	83.1
Missing	0.0	0.8	0.0	0.2	0.5	0.0	1.0
Duration (*n*)[b]	55,799	2,140	895	1,258	2,770	12,238	1,448
Years (mean ± SD)	9.4 ± 7.7	18.0 ± 11.9	17.9 ± 11.3	15.0 ± 12.0	25.3 ± 12.0	19.7±9.8	15.9±8.7
Tobacco smoking (%)							
Never	45.8	55.3	43.4	50.2	45.5	48.2	49.3
Former	17.6	31.3	34.7	41.3	36.0	30.9	30.0
Current	36.6	12.8	21.9	7.1	17.1	19.4	20.4
Missing	0.0	0.6	0.1	1.5	1.4	1.5	0.3
Alcohol intake (%)[c]							
Never		9.7	4.9	9.6	10.4	5.2	4.2
Low		28.0	24.8	29.3	28.1	31.5	27.7
Medium	N/A	28.0	24.7	29.5	30.1	31.7	27.6
High		28.1	24.7	29.4	30.0	31.6	27.7
Missing		6.3	20.9	2.2	1.5	0.0	12.9
Coffee drinking (%)							
Never					6.1	3.5	
1–3 cups per day	N/A	N/A	N/A	N/A	36.5	94.9	N/A
3+ cups per day					55.8	1.6	
Missing					1.6	0.0	
Physical activity level (%)							
0–2 h light activity per week		14.1	18.1	4.6	11.6		26.5
2+ h light activity per week		42.8	33.5	10.1	41.1		40.0
1–2 h exercise per week	N/A	25.8	21.0	70.4	35.0	N/A	16.3
2+ h exercise per week		15.8	5.4	13.0	10.6		16.6
Missing		1.4	22.0	1.9	1.7		0.6
Educational level (%)							
Compulsory		16.9	37.0	38.0	34.8	21.9	20.9
High school	N/A	42.6	46.2	36.1	35.7	53.9	56.7
University or above		39.5	15.5	23.1	23.8	23.5	22.1
Missing		1.0	1.3	2.8	5.8	0.7	0.3

N/A, not applicable; PD, Parkinson's disease; CWC, Construction Workers Cohort; SPHC, Stockholm Public Health Cohort; MONICA, Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; NMC, National March Cohort; SALT, Screening Across the Lifespan Twin Study; VIP, Västerbotten Intervention Program; WOLF, Work, Lipids and Fibrinogen Study.

Percentages may not add to 100% due to rounding.

[a]Amount, cans per week of snus use among current-users (light: < 2 cans per week, moderate-heavy: ≥ 2 cans per week).

[b]Duration, years of using snus among current-users at baseline.

[c]Alcohol intake (grams per week) was calculated from all types of alcoholic beverage, and categorized into no alcohol intake and tertiles.

per week'. Duration of using snus at baseline among current-snus users was categorized into '1 to 20 years' or 'more than 20 years' in studies where that information was available.[12,15–18]

First, we performed analyses within each cohort. Participants were followed from baseline until index date of Parkinson's disease, date of death or end of follow-up, whichever came first. To assess associations between snus use and incident Parkinson's disease in an age-adjusted model, we used Cox proportional hazards regression with attained age as the underlying time scale, estimating hazard ratios (HRs) with 95% confidence intervals (CIs). Then, we examined the associations in a multivariable-adjusted model (referred to as fully-adjusted model). Covariates included in the fully-adjusted model varied slightly between studies, based on availability of relevant information in each study. We calculated pooled estimates and 95% CIs by meta-analysing the study-specific estimates of relative risk (RR) using a random-effects model.[23] We tested for heterogeneity among the included studies, using a Q test and I^2 statistics.[24] To eliminate the potential confounding effect of tobacco smoking on the association between snus use and Parkinson's disease, we restricted the primary analyses to never-tobacco smokers, but analyses of the total sample, regardless of smoking status, were also performed. In all analyses, we used 'never-snus users' as the reference group. Further, to investigate the combined effect of snus use and tobacco smoking on Parkinson's disease risk, we cross-classified snus use and tobacco smoking according to timing of use. For this analysis, we used 'never-snus use and never-tobacco smoking' as the reference group. Last, since confounding structures for snus use and smoking might be different, we directly compared the associations for snus use versus smoking with Parkinson's disease risk. For this analysis, we categorized the snus and smoking variables into ever/never use and cross-classified the variables again, but used 'never-snus use and ever-tobacco smoking' category as the reference.

To assess the robustness of the main analyses, we conducted several sensitivity analyses. First, we excluded CWC in the meta-analyses since this cohort constituted 61.5% of the total sample size. Second, to assess the impact of missing values for potential confounders on the estimates, we ran the fully-adjusted model in each cohort by estimating missing values on all covariates with multiple imputation chain equation assuming missingness at random,[25] and then pooled study-specific estimates. Third, to examine the effect of a possible preclinical disease phase, we performed lag-time analyses by excluding the first 8 years of follow-up.[26] Fourth, to examine the extent of a possible confounding effect for covariates other than tobacco smoking (e.g. coffee and alcohol drinking), we compared the pooled HRs of SALT and VIP age-adjusted and fully-adjusted models with the primary analyses. Last, we investigated whether potential misclassifications or changes of exposure during the follow-up attenuated the association and whether there was a dose-response relationship of years of lifetime snus use with Parkinson's disease risk in the Swedish Twin Registry, in which longitudinal exposure information was available in 1974 and 1998–2002 (full details are provided in Supplementary Methods, available as Supplementary data at *IJE* online). In these analyses, exposures were treated as time-varying variables. We tested the proportional hazards assumption in the Cox model using scaled Schoenfeld residuals,[27] and no evidence of non-proportionality was found. All statistical analyses were performed with Stata, version 12 (StataCorp LP, College Station, TX).

Ethical approval

The present study was approved by the Regional Ethics Review Board in Stockholm. As the study does not include any patient's personal information, written patient consent is not required.

Results

A total of 348 601 men without a history of Parkinson's disease diagnosis at baseline were included in the study, of whom 107 838 (30.9%) reported ever-use of snus and 165 273 (47.4%) were never-tobacco smokers (Table 1). Ever-snus users were younger than never-snus users at baseline (mean ages of 37.2 and 41.4 years, respectively). There were 1199 incident Parkinson's disease cases identified (1063 only from the Patient Register, 121 from both the Patient Register and the Cause of Death Register and 15 only from the Cause of Death Register) during a mean follow-up time of 16.1 years [standard deviation (SD) 7.2]. Of the 1199 cases, 598 (49.9%) never smoked tobacco products. Mean age at Parkinson's disease diagnosis was 68.1 years for ever-snus users and 70.9 years for never-snus users.

Among never-tobacco smokers, Parkinson's disease risk in ever-snus users was lower than in never-users (pooled HR 0.41, 95% CI 0.28-0.61, $P < 0.001$ for the fully-adjusted model; Table 2). Current-snus use was associated with a lower Parkinson's disease risk than former use (Figure 2). In addition, there was evidence of dose-response relationships such that moderate-heavy amount (pooled HR 0.41, 95% CI 0.19-0.90, $P = 0.03$) and long-term current-snus users (pooled HR 0.44, 95% CI 0.24-0.83, $P = 0.01$) had the lowest Parkinson's disease risks. There was no evidence of heterogeneity between studies for the results mentioned above

Table 2. Associations between snus use and risk of Parkinson's disease among never-tobacco smokers

Snus use status	Never-tobacco smokers									
	Age-adjusted model[a]					Fully-adjusted model[b]				
				Heterogeneity					Heterogeneity	
	n	HR	95% CI	I^2	*P*-value	*n*	HR	95% CI	I^2	*P*-value
Never-users	550	1	reference			531	1	reference		
Ever-users	27	0.40	0.27-0.59	0%	0.95	27	0.41	0.28-0.61	0%	0.93
Former-users	10	0.65	0.35-1.23	0%	0.95	10	0.68	0.36-1.28	0%	0.91
Current-users	17	0.37	0.22-0.60	0%	0.89	17	0.38	0.23-0.63	0%	0.73
Amount[c]										
Light	8	0.65	0.32-1.32	0%	0.81	7	0.71	0.35-1.43	0%	0.52
Moderate-heavy	9	0.40	0.21-0.79	0%	0.38	9	0.41	0.19-0.90	18%	0.29
Per year of using snus	16	0.96	0.94-0.98	0%	0.82	16	0.96	0.94-0.98	0%	0.85
1 ~ 20 years	6	0.54	0.19-1.52	30%	0.23	6	0.56	0.19-1.68	35%	0.20
21 ~	10	0.44	0.24-0.83	0%	0.95	10	0.44	0.24-0.83	0%	0.94

n, number of Parkinson's disease cases; HR, hazard ratio; CI, confidence interval; I^2, heterogeneity statistics; *P*-value, heterogeneity Q-test.

[a]Analyses were first performed separately in each cohort among never-smokers, using Cox regression with attained age as time scale, and then pooled together using meta-analyses with a random-effects model.

[b]Adjusted for age in CWC; adjusted for age, education, alcohol and physical activity in SPHC; adjusted for age, alcohol and physical activity in MONICA; adjusted for age, alcohol and physical activity in NMC; adjusted for age, alcohol, education, physical activity and coffee intake in SALT; adjusted for age, education, alcohol and coffee intake in VIP; adjusted for age, alcohol, education and physical activity in WOLF. All analyses were conducted in never-tobacco smokers.

[c]Amount, cans per week of snus use among current-users (light: < 2 cans/week; moderate-heavy: ≥ 2 cans/week).

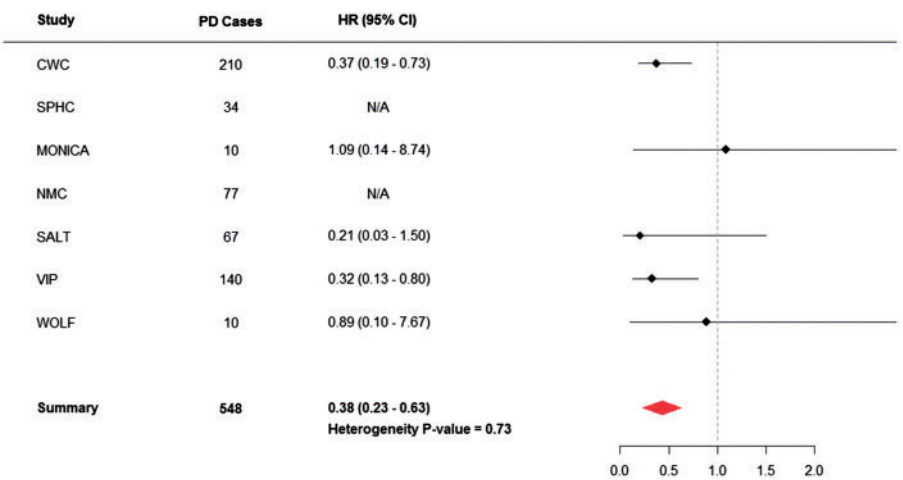

Figure 2. Study-specific and pooled estimates for the relative risk of Parkinson's disease among never-tobacco smokers comparing current-snus users versus never-snus users.

HR=hazard ratio, CI=confidence interval, PD=Parkinson's disease, N/A=Not applicable (no exposed PD cases). Analyses were first performed separately in each cohort using Cox regression with the fully-adjusted model. Study-specific estimates were then pooled together using meta-analyses with a random-effects model.

(I^2 statistics 0–35%). Analyses in the total sample regardless of smoking status showed similar results compared with the primary analyses, but with weaker associations (Supplementary Table 1, available as Supplementary data at *IJE* online). When snus use and smoking were combined, Parkinson's disease risks were lower among tobacco users, with more prominent risk reductions with current use regardless of tobacco type (Figure 3). Further, ever-snus users and never-smokers seemed to have a lower risk of Parkinson's disease, compared with never-snus users and ever-smokers (pooled HR 0.74, 95% CI 0.50-1.11, $P = 0.15$).

Sensitivity analyses left the main findings largely unchanged. In the analyses restricted to never-tobacco smokers, excluding the CWC altered the pooled HR in ever-snus users by 0.03 (Supplementary Table 2, left panel, available as Supplementary data at *IJE* online); imputation of

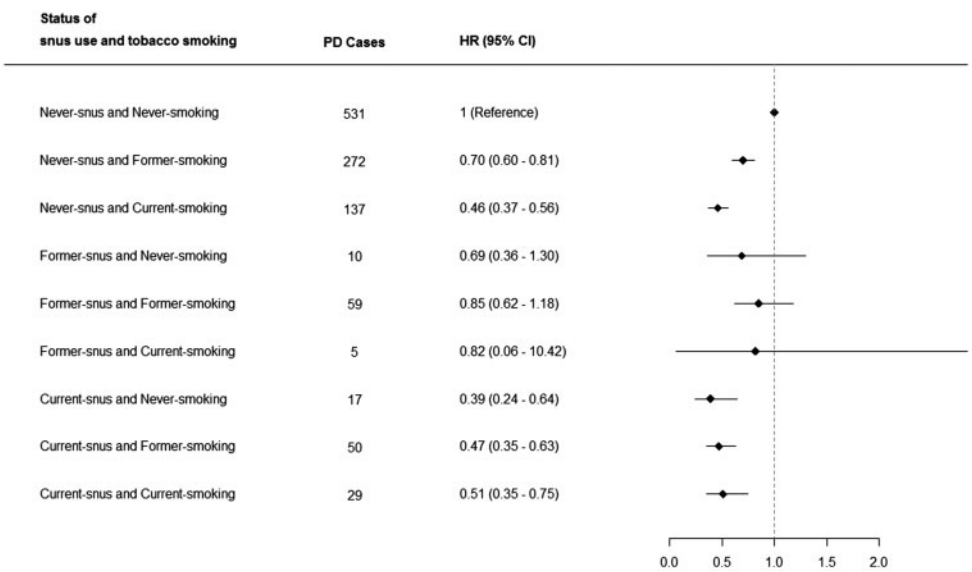

Figure 3. Combined effect of snus use and tobacco smoking on Parkinson's disease risk in seven Swedish prospective cohort studies.

HR=hazard ratio, CI=confidence interval, PD=Parkinson's disease. Analyses were first performed separately in each cohort using Cox regression with the fully-adjusted model. Study-specific estimates were then pooled together using meta-analyses with a random-effects model. Heterogeneity (P-value) for different combinations of snus use and tobacco smoking are 0.51, 0.53, 0.91, 0.26, 0.01 (former-snus users and current-tobacco smokers), 0.74, 0.74, and 0.65, respectively.

missing values on covariates altered the pooled HR in ever-snus users by 0.01 (Supplementary Table 2, middle panel); and results from lag-time analyses were similar to those of the primary analyses (Supplementary Table 2, right panel). When analyses were performed within studies with information on more covariates, differences on pooled HRs between age-adjusted and fully-adjusted models were small (Supplementary Table 3, available as Supplementary data at *IJE* online); and the fully-adjusted HR did not differ from results in the primary analyses in Table 2. When considering snus use, smoking and alcohol drinking as time-varying exposures, the associations of snus use status and longer duration of lifetime snus use with Parkinson's disease risk in the Swedish Twin Registry all had the same direction compared with the primary analyses (Supplementary Tables 4 and 5, available as Supplementary data at *IJE* online).

Discussion

This is the first pooled analysis of the relationship between smokeless tobacco use and risk of Parkinson's disease. It brought together and analysed individual participant data from seven Swedish prospective cohorts, thus compiling the largest study on Parkinson's disease and smokeless tobacco to date. We found that non-smoking men who used snus had a substantially reduced risk of Parkinson's disease. In addition, there were indications of dose-response relationships, such that risk reduction appeared more pronounced in current, moderate-heavy amount and long-term snus users. Similarly, tobacco smoking was associated with a decreased risk of Parkinson's disease, most pronounced in current smokers. Further, we examined the combined effect of snus use and tobacco smoking on Parkinson's disease risk; as expected, the reduced Parkinson's disease risks were more apparent among current-users of both types of tobacco.

To date, there is no direct evidence that nicotine, rather than other compounds from smoking, confers the protective effect on Parkinson's disease in humans. The present study addressed this issue by evaluating snus use in never-tobacco smokers for Parkinson's disease risk. The levels of nicotine concentration in blood from snus use are similar compared with smoking, but without combustion and yields of toxicants in tobacco smoke.[7] Together with the neuroprotective effect of nicotine shown in animal and *in vitro* studies,[4] our findings support the notion that nicotine can help prevent Parkinson's disease or slow the presymtomatic disease progression.

There are several lines of evidence for the possible neuroprotective properties of nicotine. Findings from *in vitro* studies suggest that nicotine inhibits α-synuclein fibrillation,[28] and that stimulation of subunits of the nicotinic-acetylcholine-receptor (nAChR) modulates inflammatory pathways.[29] In addition, there is a close anatomical relationship between nicotinic cholinergic and dopaminergic neurotransmitter systems in the striatum.[30] Extensive evidence from rodent models suggest that nicotine modulates

dopamine release by acting at nAChRs on dopaminergic nerve terminals.[31,32] Further, in non-human primates with Parkinson's disease-like motor symptoms induced by neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), chronic nicotine administration reduces striatal damage,[33] and nicotine decreases L-dopa-induced dyskinesia.[34] Last, nicotine can have a symptomatic effect in Parkinson's disease patients.[35]

An important strength of this study is that we meta-analysed data from prospective studies, thus minimizing the influence of recall bias and reverse causation. The relationship between snus use and Parkinson's disease was stable in various sensitivity analyses, and apparently not due to confounding by established Parkinson's disease risk factors. Our findings were also homogeneous among the studies included in the meta-analyses, thus suggesting high external validity. An advantage of examining the association in snus users compared with cigarette smokers is that survival bias is less likely to occur for snus use than for cigarette smoking.

Nevertheless, there are some limitations. First, although our Parkinson's disease diagnoses in the national health registers have been validated showing good accuracy,[21] this is not perfect with regard to differentiation between Parkinson's disease and other parkinsonian disorders. We believe, however, that any such misclassification is likely to be non-differential by exposure, resulting in a bias towards the null. Second, although snus use is relatively stable over time,[36] a shift to other tobacco products (e.g. cigarettes) during follow-up may have occurred. However, our sensitivity analyses with time-varying tobacco use information in the Swedish Twin Registry provided similar results compared with the primary analyses of non-smokers. Of note, it has recently been suggested that Parkinson's disease patients may experience less nicotine-mediated 'reward' from tobacco use, and therefore smoking (and snus use) cessation may be a consequence rather than a cause of Parkinson's disease.[37,38] It is also conceivable that cessation of snus use may be due to loss of dexterity in handling snus in Parkinson's disease patients not yet diagnosed. These hypotheses, unfortunately, could not be tested in the present study. Nevertheless, results from a recent study investigating the relationship between Parkinson's disease risk and time-since-cessation of smoking,[39] and our results from lag-time analyses of non-smokers, all showed significant lower Parkinson's disease risks for tobacco use, advocating a truly inverse association. Other limitations include restriction of the study population to men, and lack of possibility to adjust for all known confounders and possible unknown confounders for every study included in the meta-analyses. However, our sensitivity analyses in studies with more complete covariate information showed pooled RRs similar to the primary analyses. Although the present study pooled data from several large prospective cohorts in Sweden and results were statistically stable, larger sample size including studies from places other than Sweden would be needed to examine amount and duration of snus use, and use of other forms of smokeless tobacco, in relation to Parkinson's disease risk. Finally, other than nicotine in itself, the inverse association with Parkinson's disease could be due to a combination of nicotine and other compounds in tobacco leaf, such as monoamine oxidase inhibitors.[40]

In conclusion, data from this large pooling project showed that non-smoking men who used snus had a substantially reduced risk of Parkinson's disease. Results also indicated an inverse dose-response relationship between use of snus and subsequent risk of Parkinson's disease. Our findings hence suggest that nicotine or other components of tobacco leaves may influence the development of Parkinson's disease and explain the inverse association between cigarette smoking and Parkinson's disease risk. Further research into the role of nicotine in the prevention of Parkinson's disease is warranted.

Supplementary Data

Supplementary data are available at *IJE* online.

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Author Contributions

F.Y. performed statistical analyses, wrote the first draft and finalized the paper based on the feedback from all other co-authors. F.Y., N.L.P., K.W. and C.M. contributed to the writing of more than one draft. C.M. coordinated the collaborative pooling project. M.L. and F.Y. prepared the data. K.W., C.M., N.L.P. and F.Y. designed the study and contributed to data interpretation. All authors, external and internal, had full access to all of the data (including statistical reports and tables) in the study and critically reviewed and commented on the paper.

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