Exploring causality of the association between smoking and Parkinson’s disease

Valentina Gallo, Paolo Vineis, Mariagrazia Cancellieri, Paolo Chiodini, Roger A Barker, Carol Brayne, Neil Pearce, Roel Vermeulen, Salvatore Panico, Bas Bueno-de-Mesquita, Nicola Vanacore, Lars Forsgren, Silvia Ramat, Eva Ardanaz, Larraitz Arriola, Jesper Peterson, Oskar Hansson, Diana Gavriela, Carlotta Sacerdote, Sabina Sieri, Tilman Kühn, Verena A Katzke, Yvonne T van der Schouw, Andreas Kyrozis, Giovanna Masala, Amalia Mattiello, Robert Perneczky, Lefkos Middleton, Rodolfo Saracci and Elio Riboli

1Centre for Primary Care and Public Health, Blizard Institute, Queen Mary University of London, London, UK, 2School of Public Health, Imperial College London, London, UK, 3Epidemiology and Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London, UK, 4School of Hygiene and Preventive Medicine, University of Campania ‘Luigi Vanvitelli’, Naples, Italy, 5Hygiene and Public Health Unit, Department of Public Health, AUSL Imola, Bologna, Italy, 6Medical Statistics Unit, University of Campania ‘Luigi Vanvitelli’, Naples, Italy, 7Institute of Public Health, University of Cambridge, Cambridge, UK, 8Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands, 9Division of Epidemiology, Institute for Risk Assessment Science, Utrecht University, Utrecht, The Netherlands, 10Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy, 11National Institute for Public Health and the Environment, Bilthoven, The Netherlands, 12Department of Gastroenterology and Hepatology, University Medical Centre Utrecht, Utrecht, The Netherlands, 13Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, 14National Centre for Disease Prevention and Health Promotion, Italian National Institute of Health, Rome, Italy, 15Department of Pharmacology and Clinical Neuroscience, Umeå University, Umeå, Sweden, 16Department of Neuroscience, Psychology, Drug Research, and Child Health, University of Florence, Careggi Hospital-University, Florence, Italy, 17Navarra Public Health Institute, IdiSNA, Pamplona, Spain, 18CIBER Epidemiology and Public Health, CIBERESP, Madrid, Spain, 19Public Health Department of Gipuzkoa, Basque Government, Vitoria-Gasteiz, Spain, 20Biodonostia Research Institute, Neurosciences Area, Hospital Universitario Donostia, Donostia, Spain, 21Department of Neurology, Lund University, Lund, Sweden, 22Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Lund, Sweden, 23Department of Epidemiology, Murcia Regional Health Council, IMIB-Arrixaca, Murcia, Spain, 24Unit of Cancer Epidemiology, Centre for Cancer Prevention (CPO-Piemonte), Turin, Italy, 25Human Genetic Foundation (HuGeF), Turin, Italy, 26Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy, 27Division of Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany, 28Hellenic Health Foundation, Athens, Greece, 29First Department of Neurology, University of Athens, Athens, Greece, 30Cancer Risk Factors and Lifestyle Epidemiology Unit, Institute
Abstract

Background: The aim of this paper is to investigate the causality of the inverse association between cigarette smoking and Parkinson’s disease (PD). The main suggested alternatives include a delaying effect of smoking, reverse causality or an unmeasured confounding related to a low-risk-taking personality trait.

Methods: A total of 715 incident PD cases were ascertained in a cohort of 220 494 individuals from NeuroEPIC4PD, a prospective European population-based cohort study including 13 centres in eight countries. Smoking habits were recorded at recruitment. We analysed smoking status, duration, and intensity and exposure to passive smoking in relation to PD onset.

Results: Former smokers had a 20% decreased risk and current smokers a halved risk of developing PD compared with never smokers. Strong dose–response relationships with smoking intensity and duration were found. Hazard ratios (HRs) for smoking <20 years were 0.84 [95% confidence interval (CI) 0.67–1.07], 20–29 years 0.73 (95% CI 0.56–0.96) and >30 years 0.54 (95% CI 0.43–0.36) compared with never smokers. The proportional hazard assumption was verified, showing no change of risk over time, arguing against a delaying effect. Reverse causality was disproved by the consistency of dose–response relationships among former and current smokers. The inverse association between passive smoking and PD, HR 0.70 (95% CI 0.49–0.99) ruled out the effect of unmeasured confounding.

Conclusions: These results are highly suggestive of a true causal link between smoking and PD, although it is not clear which is the chemical compound in cigarette smoking responsible for the biological effect.

Key words: Parkinson’s disease, smoking, smoking patterns, passive smoking, causal inference, cohort study, EPIC, NeuroEPIC4PD

Key Messages

• The present data from the NeuroEPIC4PD study show a robust inverse association between smoking status at recruitment and Parkinson’s disease (PD) risk with a dose–response relationship with smoking duration and intensity.
• These inverse relationships were replicated across different clinical subtypes.
• An inverse association between exposure to passive smoking at home and/or at work and risk of PD was also identified.
• Explanation alternatives to a causal association including a delaying effect of smoking on disease onset, reverse causality, and unmeasured and residual confounding have been discussed in order to reinforce causal inference using observational data.
Background

An overwhelming amount of evidence exists on the inverse association between cigarette smoking and Parkinson’s disease (PD). The inverse association is strong and consistent across studies, stronger for current smokers than for former smokers when compared with non-smokers. Some studies suggest that smoking duration is more strongly associated with a reduced risk of PD compared with smoking intensity. The overall association appears consistent in men and women and not confounded or modified by educational level. A comparable inverse association was also observed for pipe and cigar smoking in men and for smokeless tobacco. An attempt to demonstrate causality of the association has been made using parental smoking as an instrumental variable: it was shown that children of smokers—who are more likely to smoke themselves—are at decreased risk of PD even if they do not smoke.

Nonetheless, there is still considerable caution in interpreting this association as protective. Few theories have been postulated to explain the current evidence in a non-causal way and these are summarized with Direct Acyclic Graphs (DAGs) in Figure 1. Some studies failed to replicate the association in cases with an older age of onset leading to the hypothesis that smoking might delay, not prevent, PD onset (Figure 1B). The most intriguing, and more difficult to prove, is a possible confounding effect by a low-risk-taking personality trait that would be regarded as an unmeasured confounder if it is genetically determined or as reverse causation if it is triggered by dopamine shortage (Figure 1C and D). According to this, and coherently with the involvement of dopamine in the brain-rewarding circuits, people who will subsequently develop PD tend to have a low-risk-taking personality, which makes them less likely to smoke or more likely to quit. Coherently, before disease onset, people with PD might find it easier to quit smoking compared with those without PD (Figure 1D). Nonetheless, the inverse association between smoking intensity and PD observed among monozygotic twins argues against a major role of genetics and/or personality. Given that personality trait would have a lesser role in influencing the exposure to passive smoking, demonstrating a decreased risk of PD among those exposed to passive smoking would overcome this effect; however, a previous study failed to find it.

![Figure 1](https://academic.oup.com/ije/article/48/3/912/5184917)

**Figure 1.** Direct Acyclic Graphs (DAGs) showing the hypotheses on the observed association between cigarette smoking and Parkinson’s disease. (A) Smoking protects against PD (causal effect); (B) smoking delays PD onset; (C) subjects with a specific personality trait are both less likely to smoke and more susceptible to PD (confounding effect); (D) subtle dopaminergic changes before disease onset make quitting smoking easier (reverse causality).
Clarifying the causal nature of the association between smoking and PD would contribute to understanding the mechanisms underlying the disease, informing potential targets for preventive or early treatments. Moreover, no data are currently available on the consistency of the inverse association between smoking and PD across clinical subtypes.

The aim of this study is to assess the association between smoking patterns (duration, amount and time since quitting smoking) and PD risk. Specifically, the potential delaying effect; the consistency of smoking patterns among current and former smokers to interrogate any reverse causality; the association with passive smoking; and the consistency of the association across clinical subtypes will be investigated.

Methods
Population
The NeuroEPIC study involved 220,494 subjects recruited in Sweden, the UK, the Netherlands, Germany, Spain, Italy and Greece from the general population residing in defined geographical areas between 1992 and 2002 and aged 37–70 years, within the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Exception was the Utrecht cohort, which was based on breast-cancer-screening participants. The Naples and Utrecht cohorts were restricted to women, whereas all other cohorts involved both sexes. To date, follow-up is 98.5% complete and the median follow-up time of this sample is 12.8 years [inter-quartile range (IQR) 11.5–14.2].

Case ascertainment and sample size
A total of 881 PD cases was ascertained in the participating EPIC centres. The present analysis has been conducted on a total sample of 214,533 subjects (including 715 incident PD cases) after removing 147 prevalent PD cases, 5359 subjects (including 19 PD cases) with missing information on smoking status at recruitment. Moreover, 221 subjects with PD-like conditions [Multi-System Atrophy (MSA) N = 24; Progressive Sopra-nuclear Palsy (PSP), N = 21; vascular parkinsonism, N = 34; Lewy Body Dementia (LBD), N = 34; essential tremor, N = 27; PD with essential tremor, N = 9; and unclassified parkinsonism, N = 72] were also removed from the analysis. The sample resulted in a total of 2,666,206 person/years.

Statistical analysis
Cox-regression models using age as the underlying time variable, adjusted for level of education and sex, and stratified for centre and age at recruitment, were run in order to investigate the effects of the main smoking variables in relation to PD onset. Models investigating smoking status, duration and amount of smoking, time since quitting smoking for former smokers and age when they started smoking and quit, and number of cigarettes/day smoked at different ages. This latter information was not collected in Sweden, which was therefore excluded from all analyses on smoking intensity (n = 53,291). Starting from this core information, a number of variables were derived: duration of smoking (never smokers, smokers for <20, 20–29, 30+ years) missing for 4620 individuals; smoking intensity as mean lifetime cigarettes/day (never smokers, <12, 12+ cigarettes/day) missing for 10,876 individuals; time since quitting smoking, namely number of years elapsed from quitting smoking and recruitment to the cohort (never smoker, 19+, 9–18, <9 years) missing for 2221 individuals; age when quit smoking (never smoker, <33, 34–43, 44+ years) missing for 2221 individuals; and age when started smoking (never smoker, 20+, 17–19, <16 years) missing for 301 individuals. Information on second-hand smoke (SHS) exposure was available only in a few centres: participants were asked whether any of their parents smoked when they were children in Italy, the Netherlands and Sweden (N = 59,329), whereas information on current SHS exposure at home or work was available only for participants recruited in Italy and Sweden (N = 40,816).

Additional information collected at baseline and relevant for this analysis is the highest educational level attained (none/primary, technical, secondary, university).

Smoking characteristics
Answers to a number of questions on present and past smoking habits were collected at recruitment in the EPIC study. These included smoking status at recruitment (never, former and current smoker), age when they started smoking and quit, and number of cigarettes/day smoked at different ages. This latter information was not collected in Sweden, which was therefore excluded from all analyses on smoking intensity (n = 53,291). Starting from this core information, a number of variables were derived: duration of smoking (never smokers, smokers for <20, 20–29, 30+ years) missing for 4620 individuals; smoking intensity as mean lifetime cigarettes/day (never smokers, <12, 12+ cigarettes/day) missing for 10,876 individuals; time since quitting smoking, namely number of years elapsed from quitting smoking and recruitment to the cohort (never smoker, 19+, 9–18, <9 years) missing for 2221 individuals; age when quit smoking (never smoker, <33, 34–43, 44+ years) missing for 2221 individuals; and age when started smoking (never smoker, 20+, 17–19, <16 years) missing for 301 individuals. Information on second-hand smoke (SHS) exposure was available only in a few centres: participants were asked whether any of their parents smoked when they were children in Italy, the Netherlands and Sweden (N = 59,329), whereas information on current SHS exposure at home or work was available only for participants recruited in Italy and Sweden (N = 40,816).

Additional information collected at baseline and relevant for this analysis is the highest educational level attained (none/primary, technical, secondary, university).
onset. Heterogeneity across country was tested using the approach proposed by Smith et al. Heterogeneity was assessed by the likelihood ratio of two stratified models: one with country-specific estimates and one with overall estimates. Under the null hypothesis of no heterogeneity, this statistic follows approximately a chi-square distribution on \((k - 1)^j(j - 1)\) degrees of freedom (where \(k\) is the number of categories of smoking variable and \(j\) is the total number of countries).

In order to investigate a potential delaying effect of smoking on PD onset, possible non-proportional hazard ratios was assessed using the Schoenfeld residuals. Also, the analysis on the main three smoking variables was repeated on the mid-age of PD onset after excluding subjects with an onset at 70+ years (\(<70\) years, \(N = 385\)) or on late PD onset, after excluding those with an age of onset younger than 70 years (\(70+\) years, \(N = 330\)). Studying separately subjects with a young age at onset (\(\leq 50\) years) was not possible, as there were only 12 such cases.

For indirectly exploring reverse causality, the Cox regression exploring the dose–response relationships between smoking intensity and duration were repeated among current and former smokers at recruitment separately.

Both variables on SHS (in infancy and at recruitment) where studied in relation to PD onset in Cox-regression models repeated in never smokers only in an attempt to overcome unmeasured and residual confounding of the main association.

Finally, for exploring the possible competing risk of mortality in the smoker group, a competing-risk survival analysis was carried out using death as a competing event and the Fine and Gray regression model.

A sensitivity analysis was conducted repeating the main Cox models using definite and very likely PD diagnosis only (389 PD cases). For further detail on how cases were labelled, please refer to the methodological paper. All analyses were done using STATA 12 IC and R version 3.3.2 (R Foundation for Statistical Computing, Vienna, Austria).

No direct patient involvement was needed to run this study, which was based on data previously collected.

**Results**

Demographic characteristics and smoking habits for men and women in the EPIC cohort and PD cases are described in Table 1. Former smokers at recruitment had a \(~20\%\) reduced risk of developing PD during follow-up compared with never smokers; current smokers had a halved risk compared with never smokers (Table 2). These results were highly consistent in men and women (Table 3) and no heterogeneity was detected across countries (Table 4). The difference in incidence rates across countries is more likely due to local differences in case-ascertainment procedures rather than true difference in incidence, as discussed in.

Studied individually, all smoking variables were found to be inversely associated with the risk of PD with clear-cut dose–response relationships. For age when started and quit smoking, a monotonic trend across categories was not evident (Table 2). The analysis of residuals of Schoenfeld showed no evidence of non-proportionality over the follow-up period. The smoothed curves for former smokers (Figure 2A) and for current smokers (Figure 2B) were flat, showing that beta-coefficient (log hazard ratio) estimates did not vary during follow-up (time) (Figure 2). Smoking variables were associated with inverse risk of both mid-age and late-onset PD; however, all the estimates are stronger in the latter. All the risk estimates, conversely, remain highly consistent for the akinetic-rigid and tremor-dominant forms at onset (Table 5). The Postural Instability/Gait Disturbance (PIGD) form could not be studied individually, as it included only 42 subjects.

The competing-risk analysis using mortality as a competing factor yielded much stronger point estimates but largely overlapping 95% confidence intervals (CIs) for all the active smoking variables: smoking for 30+ years or 12+ cigarettes/day is associated with a \(~55\%\) reduced risk of PD compared with never smokers (Table 2).

Hazard ratios (HRs) of smoking intensity and duration from Cox models stratified for smoking status at recruitment are shown in Figure 3. Point estimates in current smokers are consistently lower compared with those in former smokers, although the pattern of risk reduction is highly comparable across the two groups, all trends had \(p \leq 0.001\) and no interaction was detected between smoking duration and intensity and smoking status (\(p\)-value for interaction 0.823 and 0.537, respectively).

Analysis of passive smoking, although hampered by limited power, showed no association between exposure to passive smoking in infancy and risk of PD. However, an inverse association was found between passive-smoking exposure at home or at work and risk of PD (HR 0.70, 95% CI 0.49–0.99), which was replicated among never smokers only (HR 0.71, 95% CI 0.46–1.10).

The sensitivity analysis including definite and very likely PD only yielded strikingly similar results (Table 3). All associations were, if anything, strengthened despite the widening of CIs due to the smaller sample size. An inverse association between age when quitting smoking and risk of PD was also suggested by the sensitivity analysis.

**Discussion**

This study provides unique data on the inverse association between cigarette smoking and risk of PD in a large, well-
established cohort study, supporting previous findings,3,4,8 and allows testing of explanations other than a direct protective effect. Overall, data coming from the NeuroEPIC4PD study show a robust inverse association between smoking status at recruitment and PD risk, with a dose–response relationship between PD risk and smoking duration and intensity. Of particular interest is the replication of the main findings of the inverse relationship between smoking and PD among different subtypes of the disease. This is a novel finding, as, to our knowledge, clinical subtypes have not been investigated to date in such an epidemiological setting.

Table 1. Demographic characteristics and smoking habits among men and women with and without PD at recruitment in the EPIC Study

|                      | Total N = 214 533 | Men PD N = 80 389 | Women PD N = 134 144 |
|----------------------|-------------------|-------------------|----------------------|
|                      | N = 715           | N = 366           | N = 349              |
| Smoking status at recruitment |                   |                   |                      |
| Never smoker, %      | 402 (56.2)        | 149 (40.7)        | 253 (72.5)           |
| Former smoker, %     | 232 (32.5)        | 165 (45.1)        | 67 (19.2)            |
| Current smoker, %    | 81 (11.3)         | 52 (14.2)         | 29 (8.3)             |
| Duration of smoking  |                   |                   |                      |
| <20 years, %         | 92 (32.4)         | 57 (28.6)         | 35 (41.2)            |
| 20–29 years, %       | 69 (24.3)         | 47 (23.6)         | 22 (25.9)            |
| 30+ years, %         | 123 (43.3)        | 95 (47.7)         | 28 (32.9)            |
| Lifetime cigarettes/day |                 |                   |                      |
| <12 cigarettes/day, %| 91 (50.3)         | 56 (41.5)         | 35 (76.1)            |
| 12+ cigarettes/day, %| 90 (49.7)         | 79 (58.5)         | 11 (23.9)            |
| Time since quitting smoking |             |                   |                      |
| 19+ years, %         | 110 (50.7)        | 82 (52.9)         | 28 (45.2)            |
| 9–18 years, %        | 58 (26.7)         | 40 (25.8)         | 18 (29.0)            |
| <9 years, %          | 49 (22.6)         | 33 (21.3)         | 16 (25.8)            |
| Age when quit smoking |                   |                   |                      |
| <33 years, %         | 54 (24.9)         | 44 (28.4)         | 10 (16.1)            |
| 33–43 years, %       | 53 (24.4)         | 33 (21.3)         | 20 (32.3)            |
| 44+ years, %         | 110 (50.7)        | 78 (50.3)         | 32 (51.6)            |
| Age when started smoking |              |                   |                      |
| 20+ years, %         | 136 (46.0)        | 75 (36.1)         | 61 (69.3)            |
| 17–19 years, %       | 74 (25.0)         | 61 (29.3)         | 13 (14.8)            |
| <16 years, %         | 86 (29.1)         | 72 (34.6)         | 14 (15.9)            |
| Educational level    |                   |                   |                      |
| None/primary, %      | 389 (56.1)        | 192 (54.1)        | 197 (58.3)           |
| Technical, %         | 148 (21.4)        | 73 (20.6)         | 75 (22.2)            |
| Secondary, %         | 69 (10.0)         | 38 (10.7)         | 31 (9.2)             |
| University or above, % | 87 (12.6)      | 52 (14.7)         | 35 (10.4)            |
| Passive smoking      |                   |                   |                      |
| In childhood, %      | 100 (64.1)        | 36 (67.9)         | 64 (62.1)            |
| At home or at work, %| 86 (62.3)         | 34 (63.0)         | 52 (61.9)            |

*233 missing values (138 men and 85 women).
*Calculated on ever smokers only, 4620 missing values.
*Calculated on ever smokers only after excluding Swedish subjects (N = 53 291), 10 876 missing values.
*Calculated on former smokers only, 2221 missing values.
*Calculated on ever smokers only, 3011 missing values.
*Not including 2025 subjects with undetermined educational level.
*Available for 59 329 individuals only.
*Available for 40 816 individuals only.
The fact that proportional assumption hypothesis is verified demonstrates that the risk does not vary over the follow-up period, and this argues against a delaying effect of smoking on PD onset (Figure 1B). Moreover, at odds with some previous reports, our findings of an inverse relationship between smoking variables and risk of PD are not weakened when the analysis is restricted to old-age onset PD (70+ years). Taken together, these results are not supportive of the hypothesis that smoking might delay, rather than prevent, PD onset, as previously suggested. However, despite this piece of evidence being important and informative per

### Table 2. Cox-regression analyses showing hazard ratios (HRs) [and relative 95% confidence intervals (CIs)] and using as reference category never smokers or the appropriate category for each variable and HRs (and 95% CIs) for competing-risk models using mortality as competing risk

| Smoking status at recruitment | PD cases | HR (95% CI) | HR (95% CI) | Competing-risk HR (95% CI)
|------------------------------|----------|-------------|-------------|----------------------------|
| Never smokers                | 402      | 1.00        |             | 1.00                       |
| Former smokers               | 232      | 0.79 (0.66–0.94) | 0.75 (0.63–0.89) |                       |
| Current smokers              | 81       | 0.49 (0.38–0.63) | 0.44 (0.35–0.57) |                       |
| Duration of smokingb         | 402      | 1.00        |             | 1.00                       |
| Never smokers                | 92       | 0.84 (0.67–1.07) | 0.81 (0.64–1.02) |                       |
| < 20 years                   | 69       | 0.73 (0.56–0.96) | 0.67 (0.51–0.87) |                       |
| 20–29 years                  | 123      | 0.54 (0.43–0.66) | 0.49 (0.40–0.61) |                       |

**Note:** Calculated after excluding 4620 (of which 29 PD) missing values.

| Smoking intensityc           | 284      | 1.00        |             | 1.00                       |
| Never smokers                | 91       | 0.80 (0.62–1.02) | 0.77 (0.60–0.98) |                       |
| < 12 cigarettes/day          | 90       | 0.54 (0.42–0.71) | 0.49 (0.38–0.64) |                       |

**Note:** Calculated after excluding 10 876 missing values (of which 55 PD cases).

| Time since quit smokingd     | 402      | 1.00        |             | 1.00                       |
| Never smokers                | 110      | 0.87 (0.69–1.09) | 0.85 (0.68–1.06) |                       |
| 19–18 years                  | 58       | 0.71 (0.53–0.95) | 0.65 (0.49–0.87) |                       |
| < 9 years                    | 49       | 0.68 (0.50–0.93) | 0.65 (0.48–0.88) |                       |

**Note:** Calculated after excluding 54 509 (of which 96 PD cases) missing values.

| Age when quit smokingd       | 402      | 1.00        |             | 1.00                       |
| Never smokers                | 54       | 0.94 (0.70–1.26) | 0.90 (0.67–1.20) |                       |
| 34–43 years                  | 53       | 0.71 (0.52–0.95) | 0.69 (0.51–0.93) |                       |
| 44+ years                    | 110      | 0.74 (0.59–0.93) | 0.69 (0.55–0.87) |                       |

**Note:** Calculated after excluding 3011 (of which 17 PD cases) missing values.

| Age when started smokinge    | 402      | 1.00        |             | 1.00                       |
| Never smokers                | 136      | 0.74 (0.61–0.91) | 0.70 (0.57–0.85) |                       |
| 17–19 years                  | 74       | 0.59 (0.45–0.76) | 0.56 (0.44–0.72) |                       |
| < 16 years                   | 86       | 0.63 (0.49–0.81) | 0.57 (0.45–0.73) |                       |

**Note:** Calculated after excluding 3011 (of which 17 PD cases) missing values.

| Passive smoking in childhood | 56       | 1.00        |             | 1.00                       |
| Passive smoking at home/work | 86       | 0.70 (0.49–0.99) | 0.71 (0.50–1.01) |                       |

**Note:** Calculated after excluding 4620 (of which 29 PD) missing values.

**Note:** Calculated after excluding 10 876 missing values (of which 55 PD cases).

**Note:** Calculated after excluding 54 509 (of which 96 PD cases) missing values.

**Note:** Calculated after excluding 3011 (of which 17 PD cases) missing values.
se, the distinction between delaying and preventing any disease onset is somewhat artificial, as these mechanisms might coincide from both a clinical and a biological point of view.

Reverse causality

If an inverse causal relationship—accounting for subjects with a preclinical dopaminergic change who therefore might find it easier to quit smoking—was responsible for the observed inverse association between smoking and PD, the dose–response relationship between smoking duration and intensity should not hold true among former smokers (Figure 1C). The fact that the risk of PD was reduced among current and former smokers argues against this possible explanation. Furthermore, the inverse association between time since cessation and PD reinforces the idea that reverse causality is not a likely explanation of the findings: having quit smoking 9–18 years before recruitment into the study (therefore up to 30 years before disease onset) still confers a reduced risk of PD compared with never smokers.

Table 3. Hazard ratios (HRs) and relative 95% confidence intervals (CIs) from Cox-regression models investigating smoking variables in relation to PD onset in men and women separately and sensitivity analysis including only definite and very likely PD cases

| Smoking status at recruitment | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|------------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smokers                | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| Former smokers               | 165 0.77 (0.62–0.97) | | 67 0.80 (0.60–1.07) | | 121 0.85 (0.66–1.08) | |
| Current smokers              | 52 0.49 (0.35–0.67) | | 29 0.46 (0.31–0.69) | | 40 0.42 (0.29–0.59) | |

| Duration of smoking | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|---------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smokers       | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| <20 years           | 57 0.83 (0.61–1.14) | | 35 0.83 (0.58–1.21) | | 55 0.98 (0.72–1.34) | |
| 20–29 years         | 47 0.76 (0.54–1.06) | | 22 0.68 (0.43–1.07) | | 33 0.64 (0.44–0.94) | |
| 30+ years           | 95 0.55 (0.42–0.72) | | 28 0.45 (0.30–0.67) | | 64 0.52 (0.39–0.70) | |

| Smoking intensity | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|-------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smokers     | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| <12 cigarettes/day| 56 0.79 (0.57–1.10) | | 35 0.83 (0.58–1.25) | | 51 0.85 (0.61–1.19) | |
| 12+ cigarettes/day| 79 0.56 (0.42–0.76) | | 11 0.53 (0.28–0.99) | | 46 0.47 (0.33–0.68) | |

| Time since quitting smoking | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|-----------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smoker                | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| 19–33 years                 | 82 0.89 (0.67–1.18) | | 28 0.79 (0.53–1.19) | | 38 1.05 (0.77–1.44) | |
| 34–43 years                 | 40 0.68 (0.48–0.97) | | 18 0.78 (0.48–1.27) | | 28 0.67 (0.45–1.10) | |
| <9 years                    | 33 0.66 (0.45–0.97) | | 16 0.73 (0.44–1.23) | | 30 0.75 (0.50–1.11) | |

| Age when quitting smoking | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|----------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smoker               | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| <33 years                  | 44 1.10 (0.78–1.55) | | 10 0.56 (0.29–1.07) | | 36 1.25 (0.86–1.80) | |
| 34–43 years                | 33 0.60 (0.41–0.88) | | 20 0.96 (0.60–1.53) | | 28 0.74 (0.49–1.11) | |
| 44+ years                  | 78 0.72 (0.54–0.97) | | 32 0.77 (0.52–1.12) | | 52 0.73 (0.53–1.01) | |

| Age when started smoking | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|--------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| Never smoker             | 149 1.00     |             | 253 1.00       |             | 228 1.00                             |             |
| 20+ years                | 75 0.71 (0.53–0.94) | | 61 0.77 (0.57–1.04) | | 67 0.70 (0.52–0.93) | |
| 17–19 years              | 61 0.70 (0.51–0.95) | | 13 0.36 (0.20–0.64) | | 38 0.58 (0.41–0.84) | |
| <16 years                | 72 0.63 (0.47–0.84) | | 14 0.58 (0.33–1.02) | | 52 0.73 (0.53–1.01) | |

| Passive smoking in childhood | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|-----------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|
| 53 1.25 (0.70–2.24) | | 103 0.88 (0.60–1.32) | | |
| 54 0.71 (0.40–1.23) | | 84 0.68 (0.43–1.08) | | |

| Passive smoking at home/work | Men PD cases | HR (95% CI) | Women PD cases | HR (95% CI) | All Definite and very likely PD cases | HR (95% CI) |
|-----------------------------|--------------|-------------|----------------|-------------|--------------------------------------|-------------|

| Models adjusted for educational level and sex (where appropriated) and stratified by centre and age at recruitment. | Excluding Sweden (N = 53 291) and missing for 10 876 subjects who were excluded from this model. |
## Table 4. Hazard ratios (HRs) and relative 95% confidence intervals (CIs) from Cox-regression models investigating smoking variables in relation to PD onset in each country separately and p-value for heterogeneity

| Country          | PD/total | Incidence rate per 10 000 person/years | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | p-value |
|------------------|----------|----------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| Italy            | 64/40 148 | 1.32                                   | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 0.099      |
| Spain            | 101/24 924 | 3.08                                   | 1.11 (0.61–2.02) | 0.63 (0.33–1.22) | 0.91 (0.66–1.23) | 0.40 (0.11–1.48) | 0.71 (0.378–1.32) | 0.62 (0.34–1.16) | 0.74 (0.54–1.03) | 0.276 |
| UK               | 200/27 980 | 5.47                                   | 0.75 (0.38–1.48) | 0.66 (0.36–1.21) | 0.75 (0.46–1.21) | 0.27 (0.03–2.17) | 0.34 (0.14–0.84) | 0.24 (0.07–0.81) | 0.28 (0.17–0.48) | 0.060 |
| The Netherlands  | 13/16 909  | 0.73                                   | 1.58 (0.35–1.77) | 0.67 (0.29–1.51) | 0.96 (0.59–1.57) | 0.38 (0.05–3.06) | 0.79 (0.30–2.06) | 0.76 (0.32–1.77) | 0.59 (0.35–0.97) | 0.229 |
| Greece           | 92/25 845  | 20–29 years                            | 0.73 (0.37–1.45) | 0.56 (0.30–1.05) | 0.77 (0.53–1.12) | 0.38 (0.08–1.80) | 0.54–0.28–1.02) | 0.27 (0.09–0.78) | 0.31 (0.19–0.50) | 0.54 |
| Germany          | 50/25 436  | 30+ years                              | 0.276       | 0.060       | 0.229       | 0.158       | 0.070       | 0.015       | P<0.001     | 0.015       |
| Sweden           | 195/53 291 | 1.43                                   | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 0.143       | 0.397 |

| Smoking status at recruitment | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | p-value |
|-------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| Never smokers                 | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 0.099   |
| Former smokers                | 1.11 (0.61–2.02) | 0.63 (0.33–1.22) | 0.91 (0.66–1.23) | 0.40 (0.11–1.48) | 0.71 (0.378–1.32) | 0.62 (0.34–1.16) | 0.74 (0.54–1.03) | 0.276 |
| Current smokers               | 0.75 (0.38–1.48) | 0.66 (0.36–1.21) | 0.75 (0.46–1.21) | 0.27 (0.03–2.17) | 0.34 (0.14–0.84) | 0.24 (0.07–0.81) | 0.28 (0.17–0.48) | 0.060 |

| Duration of smoking | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | p-value |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| Never               | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 0.143   |
| <20 years           | 1.58 (0.81–3.11) | 0.94 (0.43–2.07) | 0.74 (0.46–1.20) | 0.33 (0.04–2.62) | 0.50 (0.15–1.67) | 0.61 (0.28–1.30) | 0.89 (0.60–1.31) | 0.297 |
| 20–29 years         | 0.78 (0.35–1.77) | 0.67 (0.29–1.51) | 0.96 (0.59–1.57) | 0.38 (0.05–3.06) | 0.79 (0.30–2.06) | 0.76 (0.32–1.77) | 0.59 (0.35–0.97) | 0.014 |
| 30+ years           | 0.73 (0.37–1.45) | 0.56 (0.30–1.05) | 0.77 (0.53–1.12) | 0.38 (0.08–1.80) | 0.54–0.28–1.02) | 0.27 (0.09–0.78) | 0.31 (0.19–0.50) | 0.015 |
| Trend               | 0.276       | 0.060       | 0.229       | 0.158       | 0.070       | 0.015       | P<0.001     | 0.015 |

| Smoking intensity   | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | p-value |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| Never               | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 1.00        | 0.397   |
| <12 cigarettes/day  | 1.08 (0.57–2.06) | 0.97 (0.53–1.77) | 0.91 (0.63–1.34) | 0.40 (0.11–1.52) | 0.60 (0.25–1.46) | 0.37 (0.15–0.91) | 0.59 (0.28–1.25) | 0.015 |
| 12+ cigarettes/day  | 0.62 (0.28–1.37) | 0.39 (0.19–0.80) | 0.68 (0.45–1.00) | –           | 0.54 (0.29–1.01) | 0.59 (0.28–1.25) | –           | –       |
| Trend               | 0.297       | 0.014       | 0.062       | 0.051       | 0.051       | 0.075       | –           | –       |

*Calculated after excluding 4620 (of which 29 PD) missing values.

*bCalculated after excluding Sweden (N = 53 291) and 10 876 missing values (of which 55 PD cases).
smokers. This results are in line with previous observational studies that showed an inverse association between parental smoking and PD in the offspring; also, the use of parental smoking as an instrumental variable overcomes the potential for a reverse-causality effect.

**Unmeasured confounding**

Whereas it was not possible to account for personality trait, its unmeasured confounding effect can be overcome by using exposure to passive smoking in relation to PD onset. Risk propensity is likely to influence one’s attitude towards active smoking, whereas passive smoking is more likely to be related to these personal characteristics in a weaker way (e.g. smokers tend to have smoking partners).

The inverse association between passive smoking and PD onset, whose point estimate has been replicated among never smokers only, argues against considering personality trait as a major confounder. These results are in line with previous reports showing how adjusting for sensation-seeking score only slightly attenuated the inverse association between smoking and PD suggesting an independent effect and with observations that personality traits such as neuroticism and introversion do not explain the inverse association between smoking and PD risk.

**Biological plausibility**

A number of substances present in tobacco have been proposed as potentially responsible for the inverse

---

Figure 2. Analysis of the residuals of Schoenfeld residuals to assess the proportionality assumption comparing former smokers (A) and current smokers (B) with never smokers. Figures represent plots of beta-coefficient estimates (log hazard ratios) for former smokers (A) and current smokers (B) against follow-up (time) in years. The darker (blue) line represents a smoothed curve of scaled Schoenfeld residuals with 95% confidence intervals (darker (blue) dotted lines), whereas the lighter (red) line represents a beta-coefficient estimate from a Cox-regression model.
association between smoking and PD. One of these is 2,3,6-trimethyl-1,4-naphthoquinone (TMN), an inhibitor of monoamine oxidase (MAO) A and B activity. TMN partially protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurodegeneration in mice by reducing endogenous dopamine metabolism and consequently decreasing oxidative stress. Synthetic MAO B inhibitors are currently used in the treatment of PD, providing symptomatic relief, but they may also protect against nigrostriatal damage decreasing dopamine metabolism, as suggested by delayed need for antiparkinsonian drugs in a recent clinical trial. Another candidate is nicotine itself, given the close anatomical relationship between the nicotinic cholinergic and dopaminergic neurotransmitter systems in the striatum. Nicotine influences also the dopaminergic activity by acting at nicotinic receptors on dopaminergic terminals and modulating dopamine release.

The role of nicotine is being investigated in a randomized trial in patients with early PD, but a role of other tobacco components cannot be excluded.

Being exposed to passive smoke is associated with a reduced risk of 30% (HR 0.70, 95% CI 0.49–0.99) and being a light smoker with a 20% reduced risk (HR 0.80, 95% CI 0.62–1.02) (Table 2). Although the difference could be due to limits in the design (data on passive smoking were available for a subset of the sample), it cannot be excluded that passive smoking has a stronger effect than one would expect from a pure equivalence of levels of exposure. Passive smoking has been demonstrated to be as mutagenic as active smoking, although earlier studies suggest that the overall chemical composition of passive smoking might not represent only the diluted composition of side-stream smoking, given the sorbing and desorbing properties of some volatile and semi-volatile organic compounds in passive smoking.

The main strengths of this study are the prospective design, the validated clinical outcome, the large sample and the detailed information on smoking patterns. This allowed a powered recall-bias-free analysis of smoking patterns in relation to PD onset. The main limitation of this study, however, is the lack of repeated smoking measurements over time, which might introduce some exposure misclassification, decreasing our ability to study smoking patterns in relation to PD onset. This is particularly true for outcomes ascertained many years after recruitment. However, the smoking pattern analyses repeated separately for PD cases ascertained within and after 8 years since recruitment yield highly consistent results (data not shown).

Conclusions

In conclusion, the present findings are consistent with a protective effect of smoking on the risk of PD. Point estimates of smoking status are strong, with a strong exposure–response relationship of smoking intensity and
duration. The consistency across different disease subtypes suggests that the putative protective effect might spread to the entire clinical spectrum of the disease. Finally, the inverse association found between passive smoking and PD is supported by a consistent finding among never smokers and points towards a true biological effect not mediated by personality type. Although smoking to prevent PD cannot be recommended given the multiple adverse effects of smoking, our results confirming an inverse association warrants further research on the mechanisms involved. In particular, the use of Mendelian randomization and biomarkers of long-term cigarette-smoke exposure should provide compelling final evidence on the inverse association between smoking and PD.

Funding
No specific funding was available for this study. The researchers are independent from any funding sources with regard to this study.

Acknowledgements
Mortality data from the Netherlands were obtained from ‘Statistics Netherlands’. In addition, we would like to thank for their financial support: Europe Against cancer Program of the European Commission (SANCO); ISCIII, Red de Centros RCESP, C03/09; Spanish Ministry of Health (ISCIII RETICC RD06/0020); Deutsche Krebshilfe; Deutsches Krebsforschungszentrum; German Federal Ministry of Education and Research; Danish Cancer Society; Health Research Fund (FIS) of the Spanish Ministry of Health; Spanish Regional Governments of Andalucia, Asturias, Basque Country, Murcia and Navarra; Spanish Ministry of Health (ISCIII RETICC.
Reference
need for additional therapies, changes in UPDRS scores, and non-motor outcomes. Lancet Neurol 2011;10:415–23.

24. Grady SR, Salminen O, Laverty DC et al. The subtypes of nicotinic acetylcholine receptors on dopaminergic terminals of mouse striatum. Biochem Pharmacol 2007;74:1235–46.

25. Quik M, Wonnacott S. alpha6beta2* and alpha4beta2* nicotinic acetylcholine receptors as drug targets for Parkinson’s disease. Pharmacol Rev 2011;63:938–66.

26. Husgafvel-Pursiainen K. Genotoxicity of environmental tobacco smoke: a review. Mutat Res 2004;567:427–45.

27. Daisey JM. Tracers for assessing exposure to environmental tobacco smoke: what are they tracing? Environ Health Perspect 1999;107:319–27.

28. Gallo V, Brayne C, Forsgren L et al. Parkinson’s disease case ascertainment in the EPIC cohort: the NeuroEPIC4PD study. Neurodegener Dis 2015;15:331–38.