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Effect of family history, occupation and diet on the risk of Parkinson disease: A case-control study

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Abstract

Background

The aetiology of Parkinson's disease (PD) is still very controversial, with a peculiar lack of established risk factors or protective behavior.

Methods

We carried out a case–control study of 634 idiopathic PD patients admitted from 2011 to 2015 to two hospitals located in central Italy and 532 controls matched by hospital, gender and age (± 5 years). The study questionnaire included questions on host factors, family history, residence, occupation and lifestyle. Odds ratios (ORs) for PD and 95% confidence intervals (CIs) were estimated with logistic regression, adjusting for actual and potential confounders.

Results

A lower OR was observed in females (0.74; 95%CI:0.58–0.96), while older age classes showed a constantly increased risk for PD (p<0.005) starting from the class 65–69 years. Subjects who reported a first degree relative affected by PD showed a borderline increase which was more evident in those enrolled in the urban center of Rome (OR = 1.65; 95%CI: 1.09–2.50). Significant reduction of the risk was associated to current smoking (OR = 0.48; 95%CI: 0.24–0.54), and to vegetables consumption (p<0.03), while borderline increases were associated to meat and cold cut consumption. Occupational activities classified according to ISCO-08 categories did not show increased risk, while higher ORs' were found for pilots and physicians.

Conclusions

The results from this study confirmed the higher risk of PD in males and in elderly, and the inverse association with smoking habit. The possible etiological role of familial clustering, dietary habit, and some job tasks is suggested.

Introduction

Parkinson disease (PD) is a neurodegenerative disorder that affects almost 6.3 million people worldwide, 1.2 million only in Europe [1]. Its pathogenesis is complex and is most likely determined by the interaction between the genetic background and environmental risk factors [2]. With the exception of few families with manifest Mendelian inheritance, preliminary evidence demonstrated a limited effect of genetic susceptibility in the etiology of PD, that needs to be deeply elucidated [2]. The relative risk (RR) of developing the diseasein the first-degree relatives of parkinsonian patients is higher than in the general population, in particular in case of early onset PD. The great variability that emerges from various studies is probably due to the mixed effect of genetic susceptibility and response to predisposing factors [3]. For this reason, the role of environmental and occupational risk factors has been extensively investigated in many epidemiological studies. An increased risk of PD has been associated in various studies with rural lifestyle, farming, pesticide exposure and also in populations with habitual residence in rural areas [4–9]. Environmental and occupational exposure to industrial chemicals, such as solvents and organo-halogenated compounds and metals, has also been related to PD [10–12].

As far as occupational risk factors are concerned, some occupations such as agricultural work [13, 14], teaching [15] and health care [13, 15] have been associated to an increased risk of PD, in addition to the above-mentioned exposure to heavy metals [16, 17], solvents [18] and pesticides [19–21]. However, definite conclusions cannot be drawn in this regard, considering also the lack of substantial support to the hypothesis that workplace factors may increase the risk of PD [22, 23]. Several other non-genetic risk factors have been investigated, mostly dealing with diet, including the oxidant/antioxidant effect of some food [24, 25].

While tobacco smoking has been inversely and consistently associated with the risk of PD in several studies [5], studies on other factors, including head injury or infectious disease, yielded inconsistent results [12].

Therefore, at present the etiology of PD is still very controversial. While the limited understanding of genetic and genomic susceptibility is common to most sporadic chronic diseases, the lack of established risk factors is quite peculiar of neurodegenerative diseases. The priority in the field of PD in order to develop successful strategies of prevention is to increase our knowledge concerning risk factor or protective behavior, to be applied in public health programs. These achievements must necessarily pass through large and comprehensive population-based case-control studies.

On these premises a large *vis-à-vis* case-control study has been conducted to investigate the role of an extensive battery of occupational, environmental, or lifestyle risk factors on the risk of PD.

Materials and methods

Study population

A study group of 548 consecutive PD patients was enrolled from the Movement Disorders out-patient clinics of the IRCCS San Raffaele Pisana hospital, located in Rome, and a second group

of 86 (13.6%) PD patients was enrolled from the San Raffaele Cassino hospital located in south of Rome in an industrial area with a vast rural hinterland. A total of 634 PD cases was included in the study. All subjects were diagnosed with idiopathic PD according to UK PDS Brain Bank Criteria [26] and were *consecutively* enrolled between December 2011 and December 2015.

Newly diagnosed subjects and patients referred for the first time to both centers were considered for inclusion after confirming the diagnosis of idiopathic PD. Subjects with suspect of secondary parkinsonism or patients not able to understand and answer the study questionnaire were excluded from enrollment. All subjects with a medical history of major psychiatric disorders, and subjects unable to understand the Italian language were excluded from the study. Controls were selected from subjects referred to the different outpatient clinics of Rome and Cassino hospitals for diagnosis other than neurodegenerative disease. Main attending clinics were the cardiological, endocrinological, orthopedic, radiological and dermatological departments. Controls were matched to PD cases by reference center and were not family members of cases. A total number of 532 controls were included in the study, 446 (83.8%) from the San Raffaele center in Rome, and 86 (16.2%) from the San Raffaele Hospital in Cassino).

Subjects that accepted to participate to the research and signed the informed consent were enrolled and administered the study questionnaire in the period between 2011 and 2015. Subjects who declined to participate (approximately 15%) did it mainly for privacy reason and lack of time. Sensitive analyses did not reveal any critical differences in demographic or clinical characteristics between subjects who refused and those that accepted to participate to the study. The protocol was designed and carried out according to the Declaration of Helsinki and approved by the IRCCS San Raffaele Pisana Ethics Committee (Prot. 12/11; 2 May 2011).

Questionnaire

All enrolled subjects were administered a questionnaire by trained personnel (MD's and research nurses). The questionnaire included information on residence history (with complete address, periods of residence and indication of rural/urban area), education (years) and occupational histories, focusing on the job tasks held during the whole of working life. All case and control subjects, according to the longest held occupations, were grouped according to the International Standard Classification of Occupations 2008 (ISCO-08) [27] in: science and engineering professionals; health professionals; teaching professionals; business and administration professionals; information and communications technology professionals; legal, social and cultural professionals; clerical support workers; services and sales workers; skilled agricultural workers; craft and related trade workers; plant and machine operators and assemblers; armed forced occupations. Housewives and subjects whose occupational data were missing were considered as separate categories. The questionnaire included also questions on the potential exposure to toxicants during leisure time activities; current and/or past substance abuse; smoking history (current/former/never; number of cigarettes; duration, years since cessation); passive smoking history (cohabiting or working with smokers, specifying the number of hours per day plus number of days per week); family history of PD/other neurodegenerative diseases (relatives/parents/siblings) together with height, weight (current and habitual) and use of diet supplements. Dietary habit prior to PD onset was assessed with a previously validated food frequency questionnaire [28] and included type and frequency of most common foods; coffee/tea/carbonated beverages drinking (frequency; amount) as well as alcohol drinking (frequency, beverage).

In addition to the general questionnaire, for selected job categories extrapolated through the preliminary analysis, a specific exposure assessment tool was developed by I.I. and V.L. to

collect additional information on the type of work activities, specific job tasks performed, workplace aspects and perceived occupational risks. The Microsoft Access database management system was used to store data and as a graphical interface for data-entry.

Statistical methods

Patients and controls characteristics were explored using descriptive statistics, and compared with univariate analysis, using student's *t* test, and χ^2 test for continuous and categorical variables, respectively. The adjusted comparison between patients with Parkinson's disease and controls was carried out through the unconditional logistic regression model [29]. Confounding effect was taken into account including in the logistic model an appropriate set of actual and potential confounders related to individual characteristics, lifestyle and dietary patterns. For each model, the occurrence of over-dispersion was checked by comparing the residual deviance with its degrees of freedom. The Likelihood Ratio Test (LRT) was applied to assess the significance of each variable in the logistic model. STATA software was used for all statistical analyses [30].

Results

The main characteristics of cases and control subjects are summarized in Table 1. Mean disease duration of cases enrolled was 7.4 ± 6.16 years (median 5.95; range: 0.005–47.07). As expected, the frequency of males was higher among cases (57.6% vs 49.1%; p<0.05). Cases were more likely to be older, never smoker, and with a relative affected by PD. The distribution of cases and controls by education, residence, and occupation classified according the ISCO-08 did not apparently show major differences. However, sub-group analysis detected that physicians and airplane pilots showed a higher proportion of cases. Clerical workers were underrepresented within cases (25.7% vs 32.9%).

The distribution of study groups by consumption of selected food items is reported in Table 2. No differences in both, the mean customary and current BMI values, were observed in the cases and in the control group. PD cases showed a lower consumption of vegetables (p<0.05), coffee (p = 0.06) and a higher consumption of meat (p<0.05), cold cuts (p = 0.014) and carbonated drinks (p = 0.043).

The results of logistic regression analysis are reported in Tables 3–5. As regards to demographic parameters, a significantly lower odds ratio (OR) was observed in females (0.74; 95% CI:0.58–0.96), while older age classes showed a constantly increased risk (p = 0.005), with statistical difference from the reference value (≤ 59 years) starting from the class 65–69 years. Subjects with higher education have generally lower, non-significant risk, and the same is observed for those living in a mixed or rural environment, i.e., 0.72 (95%CI: 0.53–0.98), and 0.97 (95%CI: 0.57–1.65) respectively. A specific item in the questionnaire investigated the presence of relatives affected by PD with a distinct question restricted to first degree relatives. Compared to those with negative family history of PD, those who reported an affected relative had a highly significant risk, OR = 2.22; 95% CI: 1.59–3.10. When the interview was restricted to first degree relatives the risk decreased to a borderline significant OR of 1.34 (95%CI: 0.92–1.93). Given the heterogeneous social background of the two clinical centers, sensitivity analyses were performed to evaluate risks by center. A significantly higher OR for first degree familiarity was found in the center located in Rome (1.65; 95%CI: 1.09–2.50).

An extensive description of the association between PD risk and various features of smoking habit is reported in S1 Table. A strong reduction of risk can be observed in current smokers, who showed a 52% lower PD risk (OR = 0.48; 95%CI: 0.33–0.69). The observed protective effect of smoking was significantly modified by the years since quitting for former smokers,

Table 1. Selected demographic and life-style characteristics of the study population by PD status (*n (%)*).

Characteristic	PD Cases (n = 634)	Controls (n = 532)	P-value
			Fisher's exact test
Sex			0.004
Males	365 (57.6)	261 (49.1)	
Females	269 (42.4)	271 (50.9)	
Age-class			0.001
≤ 59 y.	112 (17.7)	141 (26.5)	
60–64 y.	89 (14.0)	88 (16.5)	
65–69 y.	126 (19.9)	102 (19.2)	
70–74 y.	161 (25.4)	102 (19.2)	
≥ 75 y.	146 (23.0)	99 (18.6)	
Education			0.055
Primary school (5 y.)	126 (19.9)	80 (15.0)	
Secondary school(6–8 y.)	151 (23.8)	150 (28.2)	
High school(9–14 y.)	214 (33.8)	194 (36.5)	
Academic qualification (≥15y.)	140 (22.1)	103 (19.4)	
Residence			0.153
Urban	471 (74.3)	370 (69.5)	
Mixed	119 (18.8)	124 (23.3)	
Rural	36 (5.7)	30 (5.6)	
Smoking habit			<0.001
Non smoker	341 (53.8)	244 (45.9)	
Former smoker	228 (36.0)	187 (35.1)	
Current smoker	65 (10.2)	101 (19.0)	
Smoking duration			<0.001
0	341 (53.8)	244 (45.9)	
1–20	126 (19.9)	75 (14.1)	
21–40	105 (16.6)	118 (22.2)	
>40	53 (8.4)	88 (16.5)	
Pack-years			<0.001
0	341 (53.8)	244 (45.9)	
1–10	120 (18.9)	70 (13.2)	
11–30	97 (15.3)	109 (20.5)	
>30	67 (10.6)	101 (19.0)	
Passive smoking			0.292
No	167 (26.3)	154 (28.9)	
Yes	465 (73.3)	372 (69.9)	
Occupational activity			0.413
Science and engineering professionals	29 (4.7)	18 (3.4)	
Health professionals	26 (4.2)	17 (3.2)	
Teaching professionals	44 (7.1)	38 (7.2)	
Business and administration professionals	11 (1.8)	10 (1.9)	
Information and communications technology professionals	3 (0.5)	3 (0.6)	
Legal, social and cultural professionals	25 (4.0)	16 (3.0)	
Clerical support workers	160 (25.7)	173 (32.9)	
Services and sales workers	107 (17.2)	93 (17.7)	
Skilled agricultural workers	11 (1.8)	9 (1.7)	
Craft and related trade workers	65 (10.4)	43 (8.2)	

(Continued)

Table 1. (Continued)

Characteristic	PD Cases (n = 634)	Controls (n = 532)	P-value
			Fisher's exact test
Plant and machine operators and assemblers	64 (10.3)	45 (8.6)	
Armed forced occupations	11 (1.8)	4 (0.8)	
Housewives	65 (10.4)	57 (10.8)	
Prisoners	1 (0.2)	0 (0.0)	
Physicians	15 (2.4)	5 (0.9)	
Pilots	6 (0.9)	1 (0.2)	
Family history of PD *(all relatives/1st degree relatives)*			<0.001/ 0.279
No	490 (77.3)/ 551 (86.9)	467 (87.8)/ 474 (89.1)	
Yes	144 (22.7)/ 83 (13.1)	65 (12.2)/ 58 (10.9)	

although the diminishing protective effect after cessation of smoking was not homogeneous. Consistently, also parameters of smoking duration (p<0.001) and intensity (p<0.001) showed inverse trends with PD risk. No association was found with passive smoking.

The association of PD risk with the frequency of consumption of selected food items is reported in Table 4. An increasing trend can be observed with the consumption of meat. The overall LRT is not significant (p = 0.085), but the trend is significant, and those eating meat everyday showed a doubled OR when compared to those who never eat meat (OR = 1.99; 95% CI 0.95–4.17). An increased risk of PD is also reported in those drinking carbonated drinks daily (OR = 1.95; 95% CI 1.11–3.44), or even weekly (OR = 1.40; 95% CI 0.93–2.12). Cold cuts consumption exhibits an increasing trend in PD risk (p = 0.041). A protective effect has been associated to vegetable consumption (LRT p = 0.033), and to a non-significant extent to daily coffee consumption (OR = 0.80; 95% CI 0.53–1.21). No association with PD risk was found for other food items. Eventually, the risk of PD was associated to the occupational sector, as coded by ISCO-08. Using clerical support workers as referent, several sectors showed higher risks though not significant. In particular, the health professionals, with an OR of 1.59 (95% CI 0.81–3.12), plant and machine operators and assemblers (1.37; 95% CI 0.86–2.18), craft and related trade workers (1.29; 95% CI 0.81–2.06), services and sales workers (1.21; 95% CI 0.83–1.76), armed forces occupations (2.43; 95% CI 0.86–8.18). Additional analyses were performed in subgroups of these main sectors extrapolated according to the performed tasks, showing interesting results for some specific categories such as pilots (OR = 4.73; 95% CI 0.54–41.7), or reaching a significant evidence for physicians (OR = 3.07; 95% CI 1.05–9.01). Specific occupational features of these latter occupations were specifically investigated. In the group of medical doctors, neither working in laboratory or in surgery rooms, nor the distinction between working in outpatient or inpatients activities resulted associated to the risk of PD. In addition to these results, nor biological, physical, or chemical occupational risks reported by these subjects could be associated to disease onset. Similarly, logistic features, such as shift, night or overtime works did not result to influence the risk of PD in this occupational setting. No significant risk differences emerged between civil or military aircraft pilots, as well as short, medium or long-haul pilots compared to controls, and also chemical, physical, and organizational factors failed to be significantly associated with PD onset.

Discussion and conclusion

The results from the present case-control study confirmed the etiological role of some factors already described in several studies, such as the higher risk of PD for males and for older age-

Table 2. Dietary profile of the study population by PD status (*values are mean ± SD or n (%)*).

Characteristic	PD Cases (n = 634)	Controls (n = 532)	P-value
			Fisher's exact test
BMI			
Customary	25.8±3.9	25.8±4.1	0.841*
Current	26.0±4.2	26.5±4.3	0.071*
Fruit consumption			0.351
Never	1 (0.2)	4 (0.8)	
Once a month	6 (0.9)	6 (1.1)	
Once a week	93 (14.7)	67 (12.6)	
Once a day	532 (83.9)	447 (84.0)	
Vegetable consumption			0.042
Never	3 (0.5)	1 (0.2)	
Once a month	9 (1.4)	6 (1.1)	
Once a week	186 (29.3)	119 (22.4)	
Once a day	434 (68.5)	397 (74.6)	
Meat consumption			0.040
Never	26 (4.1)	28 (5.3)	
Once a month	63 (9.9)	63 (11.8)	
Once a week	486 (76.7)	407 (76.5)	
Once a day	56 (8.8)	26 (4.9)	
Fish consumption			0.549
Never	19 (3.0)	12 (2.3)	
Once a month	134 (21.1)	100 (18.8)	
Once a week	468 (73.8)	400 (75.2)	
Once a day	10 (1.6)	12 (2.3)	
Cold cuts consumption			0.014
Never	41 (6.5)	40 (7.5)	
Once a month	117 (18.5)	113 (21.2)	
Once a week	394 (62.1)	334 (62.8)	
Once a day	79 (12.5)	37 (7.0)	
Coffee consumption			0.061
Never	71 (11.2)	48 (9.0)	
Once a month	21 (3.3)	12 (2.3)	
Once a week	22 (3.5)	8 (1.5)	
Once a day	518 (81.7)	456 (85.7)	
Coffee consumption *(cups/Day)*			0.120
0–1	259 (40.9)	187 (35.2)	
2	207 (32.6)	174 (32.7)	
≥3	166 (26.2)	162 (30.4)	
Missing	2 (0.3)	9 (1.7)	
Tea consumption			0.361
Never	257 (40.5)	239 (44.9)	
Once a month	168 (26.5)	130 (24.4)	
Once a week	86 (13.6)	69 (13.0)	
Once a day	121 (19.1)	86 (16.2)	
Cola consumption			0.043
Never	321 (50.6)	297 (55.8)	
Once a month	189 (29.8)	156 (29.3)	

(Continued)

Table 2. (Continued)

Characteristic	PD Cases (n = 634)	Controls (n = 532)	P-value
			Fisher's exact test
Once a week	77 (12.1)	50 (9.4)	
Once a day	44 (6.9)	21 (3.9)	
Wine consumption			0.147
Never	138 (21.8)	141 (26.5)	
Occasionally	242 (38.2)	194 (36.5)	
Yes	253 (39.9)	194 (36.5)	
Beer consumption			0.381
Never	253 (39.9)	232 (43.6)	
Occasionally	352 (55.5)	277 (52.1)	
Yes	27 (4.3)	19 (3.6)	

*t-test.

Table 3. Association of demographic parameters with PD status (OR and 95% CIs*).

	Odds Ratio	95% Confidence Interval	P value (LRT)
Sex			0.021
Males	Ref.	-	
Females	0.74	0.58–0.96	
Age-class			0.005
≤ 59 y.	Ref.	-	
60–64 y.	1.28	0.86–1.92	
65–69 y.	1.57	1.08–2.30	
70–74 y.	1.92	1.33–2.79	
≥ 75 y.	1.76	1.21–2.57	
Education			0.376
Primary school (5 y.)	Ref.	-	
Secondary school(6–8 y.)	0.75	0.51–1.10	
High school(9–14 y.)	0.87	0.60–1.26	
Academic qualification ≥15y.)	0.98	0.65–1.48	
Residence			0.103
Urban	Ref.	-	
Mixed	0.72	0.53–0.98	
Rural	0.97	0.57–1.65	
Family history of PD (all relatives)			<0.001
No	Ref.	-	
Yes	2.22	1.59–3.10	
Family history of PD (1st degree relatives)			0.124
No	Ref.		
Yes	1.34	0.92–1.93	

*ORs and 95% CIs computed from an unconditional logistic regression model and adjusted for sex, age, smoking habit, intake of vegetable, meat, and cold cuts.

Table 4. Association of selected food items with PD status (OR and 95% CIs*).

	Odds Ratio	95% Confidence Interval	P value (LRT)	P value for trend
Meat consumption			0.085	0.028
Never	Ref.	-		
Once a month	0.93	0.48–1.80		
Once a week	1.23	0.69–2.19		
Once a day	1.99	0.95–4.17		
Cold cuts consumption			0.066	0.041
Never	Ref.	-		
Once a month	0.93	0.55–1.58		
Once a week	1.09	0.67–1.77		
Once a day	1.78	0.97–3.30		
Vegetable consumption			0.033	0.005
Never	Ref.	-		
Once a month	0.50	0.04–6.48		
Once a week	0.55	0.05–5.53		
Once a day	0.37	0.04–3.67		
Coffee consumption			0.178	0.169
Never	Ref.	-		
Once a month	1.08	0.47–2.45		
Once a week	1.76	0.70–4.39		
Once a day	0.80	0.53–1.21		
Carbonated drinks consumption			0.058	0.007
Never	Ref.	-		
Once a month	1.18	0.89–1.56		
Once a week	1.40	0.93–2.12		
Once a day	1.95	1.11–3.44		
Wine consumption			0.495	0.240
Never	Ref.	-		
Occasionally	1.22	0.88–1.68		
Yes	1.12	0.80–1.58		
Beer consumption			0.452	0.229
Never	Ref.	-		
Occasionally	1.17	0.91–1.52		
Yes	1.22	0.64–2.35		

*ORs and 95% CIs computed from an unconditional logistic regression model and adjusted for sex, age, smoking habit, intake of vegetable, meat, and cold cuts.

classes, and the inverse association with smoking habit. On the other hand, the availability of a comprehensive questionnaire which included host factors, residence, occupation, and several features of lifestyle, allowed to study a number of potential risk factors insufficiently investigated so far. This approach suggested that a family history of PD, the consumption of meat, cold cuts and carbonated drinks, working as a medical doctor or as a pilot increased the risk of PD, while a constant consumption of vegetables reduced the risk, especially in those eating vegetables daily. The negative association with vegetable consumption were quantitatively unstable due to the small number of subjects in the reference category of those who never have vegetables. A more balanced comparison of subjects who eat vegetables everyday vs those who have vegetables once per week confirmed the trend reported in Table 5 (OR = 0.69, p<0.01).

Table 5. Association of selected sectors of occupation with PD status (OR and 95% CIs*).

Occupational activity	PD Cases (n = 634)	Controls (n = 532)	Odds Ratio	95% Confidence Interval	P value (LRT)
					0.893
Clerical support worker	160	173	Ref.	-	
Science and engineering professionals	29	18	1.34	0.69–2.61	
Health professionals	26	17	1.59	0.81–3.12	
Teaching professionals	44	38	1.33	0.79–2.23	
Business and administration professionals	11	10	0.85	0.34–2.14	
Information and communications technology professionals	3	3	1.36	0.26–7.01	
Legal, social and cultural professionals	25	16	1.33	0.67–2.64	
Services and sales workers	107	93	1.21	0.83–1.76	
Skilled agricultural workers	11	9	1.04	0.41–2.67	
Craft and related trade workers	65	43	1.29	0.81–2.06	
Plant and machine operators and assemblers	64	45	1.37	0.86–2.18	
Armed forced occupations	11	4	2.43	0.73–8.13	
Housewives	65	57	1.25	0.78–2.00	
Physicians	15	5	3.07	1.05–9.01	
Pilots	6	1	4.73	0.54–41.7	

*ORs and 95% CIs computed from an unconditional logistic regression model and adjusted for sex, age, smoking habit, intake of vegetable, meat, and cold cuts.

The most convincing evidence from our study concerned the inverse association with smoking habit [31, 32]. In agreement with existing literature the protective effect of smoking was higher in those subjects with a long history of smoking (OR = 0.36; 95% CI 0.24–0.54 in those smoking 40 years or more) and in those smoking more than 30 pack-years (OR = 0.40; 95% CI 0.27–0.58), while no consistent results were found in the group of former smokers with the years since cessation. Altogether, these results, in association with those referring to the effect of gender and age confirm the high degree of internal validity of this study, an important condition that increases the credibility of innovative findings referring to other possible risk factors. As concerns other voluptuary habits, i.e. the impact of alcohol assumption on PD risk, no consistent pattern was found in our data, reflecting the extreme variability of results from the literature [24, 33], characterized by the heterogeneity of methods used to assess alcohol consumption, the genetic variability of population settings, and the contrasting inclusion criteria.

The presence of an inherited predisposition to PD has been the object of several investigations, as extensively reported by Thacker and Ascherio [3]. As regards our results we clearly showed that the use of non-first-degree-only relatives results in a biased approached, due to tendency of individuals with PD to be more likely aware of PD diagnosis in higher-order relatives than controls, i.e., OR = 2.22 vs 1.34, respectively. On the other hand, our approach, based on a *vis-à-vis* contact, a reconstruction of the family composition (number of children), and an estimate of OR for subjects with positive family history adjusted for a large battery of predictive factors, supports the conclusion that the increased probability of PD in subjects positive for familial history of PD may have been overestimated. The adjusted estimation procedure yields in a level of risk which is in the same range of other major multifactorial diseases, such as cancer [34] and cardiovascular diseases [35]. The confirmation of how several factors may change the strength of the association between positive familial history of PD and individual risk comes from the difference between the two clinical centres contributing cases and controls to this study. The higher and significant risk found in subjects from the centre in Rome

(OR = 1.65; 95%CI: 1.09–2.50), given the standard methods used in the assessment, supports the hypothesis that a shared environment or a population setting may modify the level of inherited susceptibility.

As regards the different components of the shared environment there is compelling evidence that a proper diet can promote healthy aging and prevent certain environmental diseases, although whether this would be the case in PD is still controversial. Previous studies have presented inconsistent results concerning the association between PD risk and the consumption of different meats and vegetables [24]. In our study PD cases showed a lower consumption of vegetables and a higher consumption of meat and cold cuts; the protective effect of caffeine has been confirmed [36], although the high coffee consumption in Italy (83.6% of the whole study group had one or more cups per day) has most likely reduced the strength of the association. Interestingly these results overlap those of other major multifactorial diseases, including cancer, cardiovascular and other inflammatory diseases [34, 35], supporting the view that the benefits of a healthy dietary pattern reflect the synergies and cumulative effects of different nutrients, rather than being the result of a single nutrient.

The effect of work on the risk of PD is relatively understudied and poorly understood. Most previous studies of occupation and PD have focused on exposures to toxicants (e.g., pesticides, solvents, lead, welding fume, and electromagnetic fields) or low socioeconomic status occupations, such as farming, construction, production, and military service [12, 13, 18]. More recently higher socio-economic status occupations have been investigated (e.g., computer and mathematical; architecture and engineering; legal; and education, training, and library occupations) [37], although general belief is that the role of occupation in the aetiology of PD is minimal. The general results of our study are in agreement with this conclusion, as we can find in a number of occupational activities such as health professionals, plant and machine operators/assemblers, craft and related trade workers, services and sales workers as well as armed forced employees, only a borderline increased risk for PD. On the other hand, subgroup analysis demonstrated that specific job titles may be associated with PD, such as in the case of pilots and physicians. Actually, the increased risk for PD in the medical profession (OR = 3.07; 95% CI 1.05–9.01 in our study) has been previously described in literature [13, 15] and it has been usually associated with a greater exposure of this category to infectious agents, supporting the "neuro-inflammatory" hypothesis in the PD pathogenesis [38, 39]. Despite the careful exposure assessment performed in our study (all 20 medical doctors were re-interviewed using an *ad hoc* questionnaire on their work) no definite conclusions can be currently extrapolated concerning possible occupational factors, in terms of specific tasks performed, perceived workplace risks as well as logistic factors, influencing such association. This lack of evidence may in part be due to the limited number of investigated subjects in each subgroup and requires that more detailed occupational investigation should be performed based on specific job task-exposure assessment and the interpretation of underlined mechanisms of action. The possible exposure to biological and chemical agents, ionizing or non-ionizing radiations, shift-works, sedentary works or workload activities as well as job strain may act as triggers for chronic mild inflammatory status in the *substantia nigra*, which makes dopaminergic neurons vulnerable to degeneration. All these aspects should be carefully verified in future *ad hoc* multi-centric, epidemiological investigations based on larger homogeneous groups of workers. An interesting hypothesis suggests that the early deterioration of dopaminergic pathways producing subtle, sub-clinical effects long before the onset of symptoms, may induce character changes addressing future patients towards working careers with particular features, i.e. less physical fatigue, high demand for precision [40].

The present study has several strengths, including the prospective collection of cases, the large sample size, which makes this study among the largest with direct interview to cases and

controls, the comprehensive questionnaire covering host factors, life-style, diet and occupation, which allowed to estimate adjusted OR's from regression models with the best goodness-of-fit. Obviously, the study has also a number of limitations. The presence of recall bias by subjects affected by PD, for those variables that may have been associated to the disease, i.e. familiarity and occupation, was addressed restricting to first degree relatives the familial relationships and performing a dedicated exposure assessment for those occupations which resulted associated to PD. As regards dietary habit, due to the limited evidence available, there is not a common believe that diet may be associated to the risk of developing PD, and therefore the presence of recall bias it is quite unlikely. On the other hands, since the questionnaire investigated dietary habits prior to PD diagnosis, a long-term recall bias cannot be ruled out. The study was performed in two different clinical centers. Despite the common geographical location in the center of Italy, the two centers differed for the socioeconomic status of the two populations, a condition which may have determined an incomplete adjustment for confounding. A general limitation of the study is also the lack of specific analyses concerning Parkinson's disease subtypes, which in some papers have been reported to have a differential sensitivity to potential risk factors [14]. Additional re-analysis of data based on clinical PD subtypes may provide a critical improvement of current findings. The presence of numerous hypotheses tested may have generated a multiple comparison issue. Due to this condition, some exposure could have been identified as significantly associated to PD just by chance, and their p value may be unreliable. A correction was not applied since all covariates associated to the risk of PD were previously known as possible risk factors or had a strong mechanistic background that supported the results.

In conclusion, this study has confirmed some of the associations already present in literature but has also highlighted an increased risk for PD in some peculiar occupational activities and an important link to diet that could open the door to new lines of research. Furthermore, from our results clearly emerges that to understand the complexity of exposures faced throughout the lifespan, emerging techniques of biomonitoring should be integrated, with the aim of identifying strategies to transition exposure research toward exposomics and to start modelling a holistic strategy of prevention, based on genomics, clinical features, personality, lifestyle, aging, and comorbidities.

Supporting information

S1 Table. Association of different indexes of smoking habit with PD status (OR and 95% CIs*). *ORs and 95% CIs computed from an unconditional logistic regression model and adjusted for sex, age, intake of vegetable, meat, and cold cuts. ** Adjusted also by active smoke. (DOC)

S1 Dataset. Dataset generated during the study.
(XLSX)

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References

1. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014 Nov; 29(13):1583–90. https://doi.org/10.1002/mds.25945 PMID: 24976103

2. Lesage S., Brice A. Parkinson's disease: From monogenic forms to genetic susceptibility factors. Hum. Mol. Genet. 2009; 18:R48–R59. https://doi.org/10.1093/hmg/ddp012 PMID: 19297401

3. Thacker EL and Ascherio A. Familial Aggregation of Parkinson's Disease: A Meta-Analysis. Mov Disord. 2008; 23: 1174–83. https://doi.org/10.1002/mds.22067 PMID: 18442112

4. Nandipati S, Litvan I. Environmental Exposures and Parkinson's Disease. Int J Environ Res Public Health. 2016; Sep 3: 13(9). https://doi.org/10.3390/ijerph13090881 PMID: 27598189

5. Breckenridge CB, Berry C, Chang ET, Sielken RL Jr, Mandel JS. Association between Parkinson's Disease and Cigarette Smoking, Rural Living, Well-Water Consumption, Farming and Pesticide Use: Systematic Review and Meta-Analysis. PLoS ONE. 2016; 11(4): e0151841. https://doi.org/10.1371/journal.pone.0151841 PMID: 27055126

6. Weisskopf MG, Knekt P, O'Reilly EJ, Lyytinen J, Reunanen A, Laden F, et al. Persistent organochlorine pesticides in serum and risk of Parkinson disease. Neurology. 2010; 74: 1055–61. https://doi.org/10.1212/WNL.0b013e3181d76a93 PMID: 20350979

7. Priyadarshi A, Khuder SA, Schaub EA, Shrivastava S. A meta-analysis of Parkinson's disease and exposure to pesticides. Neurotoxicology. 2000; 21: 435–40. PMID: 11022853

8. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE, et al. Pesticide exposure and risk for Parkinson's disease. Ann Neurol. 2006; 60:197–203. https://doi.org/10.1002/ana.20904 PMID: 16802290

9. Brouwer M, Huss A, van der Mark M, Nijssen PCG, Mulleners WM, Sas AMG, et al. Environmental exposure to pesticides and the risk of Parkinson's disease in the Netherlands. Environ Int. 2017; 107:100–10. https://doi.org/10.1016/j.envint.2017.07.001 PMID: 28704700

10. Caudle WM, Guillot TS, Lazo CR, Miller GW. Industrial toxicants and Parkinson's disease. Neurotoxicology. 2012; 33(2): 178–88. https://doi.org/10.1016/j.neuro.2012.01.010 PMID: 22309908

11. Caudle WM, Guillot TS, Lazo C, Miller G W. Parkinson's disease and the environment: Beyond pesticides. Neurotoxicology. 2012; 33(3): 585. https://doi.org/10.1016/j.neuro.2012.04.011 PMID: 22774228

12. Lai B C L, Marion SA, Teschke K, Tsui JKC. Occupational and environmental risk factors for Parkinson's disease. Parkinsonism & related disorders. 2002; 8(5): 297–309. https://doi.org/10.1016/s1353-8020(01)00054-2 PMID: 15177059

13. Park J, Yoo CI, Sim CS, Kim HK, Kim JW, Jeon BS, et al. Occupations and Parkinson's disease: A multi-center case-control study in South Korea. Neurotoxicology. 2005; 26:99–105. https://doi.org/10.1016/j.neuro.2004.07.001 PMID: 15527877

14. Moisan F, Spinosi J, Delabre L, Gourlet V, Jean-Louis Mazurie JL, Bénatru I, et al. Association of Parkinson's Disease and Its Subtypes with Agricultural Pesticide Exposures in Men: A Case–Control Study in France. Environ Health Perspect. 2015; 123:1123–29. https://doi.org/10.1289/ehp.1307970 PMID: 25815770

15. Tsui JK, Calne DB, Wang Y, Schulzer M, Marion SA. Occupational risk factors in Parkinson's disease. Can J Public Health. 1999; 90:334–37. https://doi.org/10.1007/BF03404523 PMID: 10570579

16. Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, et al. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Neurotoxicology 1999; 20:239–47. PMID: 10385887

17. Lan AP, Chen J, Chai ZF, Hu Y. The neurotoxicity of iron, copper and cobalt in Parkinson's disease through ROS-mediated mechanisms. Biometals. 2016; 29(4):665–78. https://doi.org/10.1007/s10534-016-9942-4 PMID: 27349232

18. McDonnell L, Maginnis C, Lewis S, Pickering N, Antoniak M, Hubbard R, et al. Occupational exposure to solvents and metals and Parkinson's disease. Neurology. 2003; 61:716–17. https://doi.org/10.1212/wnl.61.5.716 PMID: 12963777

19. Frigerio R, Sanft KR, Grossardt BR, Peterson BJ, Elbaz A, Bower JH, et al. Chemical exposures and Parkinson's disease: A population-based case-control study. Mov Disord. 2006; 21:1688–92. https://doi.org/10.1002/mds.21009 PMID: 16773614

20. Kamel F, Tanner C, Umbach D, Hoppin J, Alavanja McR, Blair A, et al. Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. Am J Epidemiol. 2007; 165: 364–374. https://doi.org/10.1093/aje/kwk024 PMID: 17116648

21. Valdés EG, Andel R, Sieurin J, Feldman AL, Edwards JD, Långström N, et al. Occupational complexity and risk of Parkinson's disease. PloSone 2014; 9(9):e106676. https://doi.org/10.1371/journal.pone.0106676 PMID: 25198429

22. Firestone JA, Lundin JI, Powers KM, Smith-Weller T, M Franklin G, Swanson PD, et al. Occupational factors and risk of Parkinson's disease: A population-based case-control study. Am J Ind Med. 2010; 53(3):217–23. https://doi.org/10.1002/ajim.20788 PMID: 20025075

23. van der Mark M, Vermeulen R, Nijssen PC, Mulleners WM, Sas AMG, van Laaret T, et al. Occupational exposure to solvents, metals and welding fumes and risk of Parkinson's disease. Parkinsonism RelatDisord. 2015 Jun; 21(6):635–9. https://doi.org/10.1016/j.parkreldis.2015.03.025 PMID: 25903042

24. Erro R, Brigo F, Tamburin S, Zamboni M, Antonini A, Tinazzi M. Nutritional habits, risk, and progression of Parkinson disease J Neurol 2018; 265:12–23. https://doi.org/10.1007/s00415-017-8639-0 PMID: 29018983

25. Qi H, Li S. Dose-response meta-analysis on coffee, tea and caffeine consumption with risk of Parkinson's disease. Geriatr Gerontol Int 2014: 14:430–39. https://doi.org/10.1111/ggi.12123 PMID: 23879665

26. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease. A clinico-pathological study of 100 cases. JNNP 1992; 55:181–84. https://doi.org/10.1136/jnnp.55.3.181 PMID: 1564476

27. International Standard Classification of Occupations ISCO-08, Vol. 1. Structure, Group definitions and Correspondence Tables. International Labour Office, Geneva; ILO, 2012. (http://www.ilo.org/wcmsp5/groups/public/—dgreports/—dcomm/—publ/documents/publication/wcms_172572.pdf. December 2017

28. Fenech M, Baghurst P, Luderer W, et al. Low intake of calcium, folate, nicotinic acid, vitamin E, retinol, beta-carotene and high intake of pantothenic acid, biotin and riboflavin are significantly associated with increased genome instability—results from a dietary intake and micronucleus index survey in South Australia. Carcinogenesis. 2005; 26(5):991–999. https://doi.org/10.1093/carcin/bgi042 PMID: 15705599

29. JL Fleiss B Levin MC Paik. Statistical Methods for Rates and Proportion. 3rd ed. 2003. John Wiley & Sons, Inc. Hoboken, New Jersey

30. StataCorp. (2015) Stata Statistical Software: Release 14. College Station, TX: StataCorp LP

31. van der Mark M, Nijssen PC, Vlaanderen J, Huss A, Mulleners WM, Sas AMG et al. A case-control study of the protective effect of alcohol, coffee, and cigarette consumption on Parkinson disease risk: time-since-cessation modifies the effect of tobacco smoking. PLoS One. 2014; 9(4):e95297. https://doi.org/10.1371/journal.pone.0095297 PMID: 24788751

32. Breckenridge CB, Berry C, Chang ET, Sielken RL Jr, Mandel JS. Association between Parkinson's Disease and Cigarette Smoking, Rural Living, Well-Water Consumption, Farming and Pesticide Use: Systematic Review and Meta-Analysis. PLoS One. 2016; 11(4):e0151841. https://doi.org/10.1371/journal.pone.0151841 PMID: 27055126

33. Zhang D, Jiang H, and Xie J. Alcohol intake and risk of Parkinson's disease: a meta-analysis of observational studies. Mov Disord. 2014; 29(6): 819–22. https://doi.org/10.1002/mds.25863 PMID: 24590499

34. Coté ML, Liu M, Bonassi S, Neri M, Schwartz AG, Christiani DC, et al. Increased risk of lung cancer in individuals with a family history of the disease: a pooled analysis from the International Lung Cancer Consortium. Eur J Cancer. 2012; 48(13):1957–68. https://doi.org/10.1016/j.ejca.2012.01.038 PMID: 22436981

35. Nielsen LA, Nielsen TR, Holm JC. The Impact of Familial Predisposition to Obesity and Cardiovascular Disease on Childhood Obesity. Obes Facts. 2015; 8(5):319–28 https://doi.org/10.1159/000441375 PMID: 26465142

36. Wood H. Parkinson disease: Caffeine and nicotine do not provide symptomatic relief in Parkinson disease Nat Rev Neurol. 2017; 13(12):707.

37. Beard J, Steege AL, Ju J, Lu J, Luckhaupt SE, Schubauer-Berigan MK. Mortality from Amyotrophic Lateral Sclerosis and Parkinson's Disease Among Different Occupation Groups—United States, 1985–2011. MMWR 2017; 66:718–22. https://doi.org/10.15585/mmwr.mm6627a2 PMID: 28704346

38. McGeer PL, Mc Geer EG. Inflammation and neurodegeneration in Parkinson's disease. Parkinsonism Relat Disord. 2004; 10(suppl 1):S3–S7. https://doi.org/10.1016/j.parkreldis.2004.01.005 PMID: 15109580

39. Whitton PS. Inflammation as a causative factor in the aetiology of Parkinson's disease. Br J Pharmacol. 2007; 150:963–76. https://doi.org/10.1038/sj.bjp.0707167 PMID: 17339843

40. Santangelo G, Piscopo F, Barone P, Vitale C. Personality in Parkinson's disease: Clinical, behavioural and cognitive correlates. J Neurol Sci. 2017 Mar 15; 374:17–25. https://doi.org/10.1016/j.jns.2017.01.013 PMID: 28087060