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The relationship between environmental factors and different Parkinson's disease subtypes in Greece: Data analysis of the Hellenic Biobank of Parkinson's disease

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ABSTRACT

Introduction: The aim of this study is to investigate the association between environmental factors (smoking, coffee, pesticide exposure) and Parkinson's disease (PD) subtypes (early-onset, mid-and-late onset, familial and sporadic) in the Greek population.

Methods: The Hellenic Biobank of PD recorded information of PD cases and controls from two centers in Greece during 2006–2017. Patients with the A53T mutation in *SNCA* or *GBA* mutations were excluded. Associations of environmental factors with PD overall (and PD subtypes) versus controls were explored with logistic regression models adjusting for age, gender and each environmental factor.

Results: 686 patients and 356 controls were included. Smoking was associated with a reduced risk of PD overall (OR 0.48, 95% CI 0.35–0.67), mid-and-late onset (0.46, 0.32–0.66), familial (0.53, 0.34–0.83) and sporadic (0.46, 0.32–0.65), but not early-onset PD. There was an inverse linear association with pack-years of smoking, except for early-onset PD. Early-onset PD was the only PD subtype inversely associated with coffee consumption when dichotomously treated. Compared to never-coffee drinkers, only those at the upper tertile had lower odds for PD overall (0.52, 0.29–0.91), early-onset (0.16, 0.05–0.53) and familial PD (0.36, 0.17–0.75). No associations were found between pesticides and PD.

Conclusions: Our study shows that the well-known negative association of smoking with PD occurs across all PD subtypes in the Greek population, apart from early-onset PD. Early-onset PD was also most strongly inversely associated with coffee consumption, highlighting a potential distinct underlying physiopathology in this PD subset that may involve specific gene-environment interactions.

1. Introduction

Parkinson's disease (PD) is considered to be a multifactorial disorder with unknown etiology, since a complex interaction between genetic factors, such as *SNCA*, *Parkin*, *LRRK2* and *GBA* gene mutations, and environmental exposures contribute to its development [1]. In particular, many epidemiological studies and their meta-analyses have revealed that smoking and coffee intake may be inversely associated with PD development [2,3], whereas exposure to pesticides could increase PD risk [4]. However, the sample size of some epidemiological studies in this field is relatively small and the results of many of them are rather conflicting. Furthermore, most of the relevant studies have not taken into account the presence of specific SNPs or gene mutations, whose interaction with specific environmental factors could possibly alter PD risk [5]. PD is a clinically heterogeneous disorder, and a complicated interplay between genetic and environmental factors could contribute

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to this diversity. Several PD classifications into specific subtypes have been proposed, including i) early-vs mid-and-late-onset PD, ii) familial vs sporadic PD, iii) tremor dominant vs akinetic rigid vs mixed PD and iv) mainly motor/slow progression vs intermediate vs diffuse/malignant PD [6]. There is still no consensus regarding the best PD classification scheme, whereas the specific aims of each study would rather define the most appropriate one. Although there is still no consistent definition about early-onset PD, the onset of symptoms before the age of 50 is defined as early-onset PD by most studies [7]. However, it should be noted that this subtype classification is not well validated, and the cut-off point of 50 years is somewhat arbitrary. Early-onset PD patients display higher rates of non-motor symptoms and motor complications, as well as a slower rate of disease progression in several epidemiological studies [7], suggesting that early-onset PD could represent a PD subtype with distinct pathophysiologic background. Furthermore, familial forms of PD, usually defined as the presence of at least one first- or second-degree relative with PD in epidemiological studies and probably reflecting an inherited susceptibility, have been considered as a different PD subcategory, given the fact that genetic factors have been associated with specific phenotypes and disease course [8]. However, phenotypic variation within patients in the same family suggests additional gene-environmental interactions that could alter PD risk. In this context, there is an ongoing uncertainty regarding whether various forms of PD (early-onset PD, mid-and-late onset PD, familial PD and sporadic PD) are related to different environmental exposures [9]. Indeed, most epidemiological studies fail to assess associations in such PD subtypes, and rather consider PD as a homogenous disorder. Epidemiological data on PD in Greece are scant. Given also the fact that ethnicity has been shown to at least partially contribute to clinical PD variability [10], we sought to investigate the associations between environmental factors and PD development in separate analyses for the abovementioned PD subtypes in the Greek population.

The Hellenic Biobank of Parkinson's Disease (HBPD) stores blood samples, as well as genetic, medical and lifestyle information about PD patients and control subjects who visited two centers in Greece, during 2006–2017. Data collection is still ongoing. Based on the existing data in the HBPD, in the current work we aimed to investigate the association between environmental factors (smoking, coffee intake and pesticide exposure) and the development of different PD subtypes (early-onset PD, mid-and-late onset PD, familial PD, sporadic PD) in the Greek population. Given the fact that early onset and familial cases both have an increased genetic component that drives PD in their case, we also aim to pave the way for the future investigation of potential gene-environmental interactions affecting PD risk.

2. Methods

2.1. Patients and controls

Our data were obtained from the HBPD. The HBPD includes information about PD patients and controls who were recruited between 2006 and 2017 from the General Hospital of Syros and the Attikon University Hospital in Athens. PD diagnosis was made by Movement Disorder specialists (MB, MS, LS), and was based on the criteria for probable or possible PD proposed by Gelb and colleagues [11]. We excluded patients with other causes of parkinsonism, such as PSP, MSA and pharmaceutical or vascular parkinsonism via the use of brain MRI or DAT scan for patients with unclear or atypical clinical presentation. The present study was conducted in agreement with the principles of the Declaration of Helsinki. Signed informed consent was obtained from all participants recruited. The study was approved by the Scientific Board of Attikon Hospital. Control subjects were without PD diagnosis, with no family history of PD up to second degree relatives. They were usually caregivers of PD patients, or outpatients visiting the Hospital for other unrelated non-neurological complains. Care was taken to ensure that cases and controls did not differ remarkably in terms of age and were recruited from generally the same geographical areas. There were no other specific exclusion criteria. Controls were enrolled at the same time in HBPD as the patients with PD.

The enrollment procedure included demographic, clinical and lifestyle information obtained through questionnaire interviews, as well as blood sample collection for DNA testing. Genetic testing for the p.A53T mutation in the SNCA gene was conducted in patients with onset age of PD ≤ 50 years or a positive family history of PD. In the case of negative results of SNCA testing, GBA mutations were also examined. Patients found to be positive for A53T or GBA mutations were excluded from our analysis, in order to minimize genetic bias. We elected not to study separately these genetic forms in this epidemiological study due to the fact that their numbers, for the GBA and SNCA cohorts, are quite low.

2.2. Data collection

DNA was extracted from peripheral blood of the patients and was analyzed for the p. A53T *SNCA* mutation, as described previously by Bozi and colleagues [12] and for *GBA* mutations as described by Moraitou and colleagues [13].

Information about exposure to environmental factors and family history of PD was obtained at the time of the enrollment in HBPD through specific questionnaire interviews administered by trained medical doctors. Cigarette smoking was self-assessed with the following question: “Have you smoked more than 100 cigarettes throughout your life?“. Coffee consumption was determined with the question: “Have you consumed coffee in the past?” and exposure to pesticides with the question: “Have you been ever exposed to pesticides?“. The participants provided a “yes/no” answer for all these questions. Quantitative data were also obtained, regarding duration (years) and intensity of smoking and coffee consumption (average number of cigarettes smoked/cups of coffee consumed per day respectively), as well as the duration of pesticide exposure. Information about the type of pesticide exposure (indirect, gardening or occupational) was also collected.

All patients were given a structured neurological examination by movement disorder specialists at the time of the enrollment in HBPD, on the same day with blood sample collection and interviews. Onset age of PD was defined as the self-reported age at which the first cardinal symptom was noticed. We used the cutoff of 50 years for early-onset PD like most relevant studies [7]. A positive family history of PD was defined as the occurrence of at least one first or second-degree family member with a diagnosis of PD.

2.3. Statistical analysis

All data were analyzed using IBM SPSS Statistics version 22. All qualitative variables were treated as dichotomous (yes/no). Quantitative data (e.g. age, smoking years, daily cups of coffee consumed) underwent a normality (Kolmogorov-Smirnov) test. For not-normally distributed variables non-parametric Mann-Whitney *U* test was applied. In order to investigate gender differences between groups of missing and non-missing values regarding environmental factors Fisher exact test was applied. Values less than 0.05 were considered significant.

Initially, we assessed the association between each of the examined environmental factors (cigarette smoking, coffee consumption and pesticide exposure; all dichotomously coded) and PD outcome (all PD patients versus Controls), adjusted for age, gender and each of the other environmental factors in one model, so that the results can be also directly compared to prior relevant studies using ever/never [14].

Next, we calculated smoking pack-years and coffee cup-years for each case. We considered the non-smoking and non-coffee drinking as the reference categories. Then we grouped smokers and coffee drinkers into tertiles that we called “light”, “moderate” and “heavy” exposure groups (Table 1). We performed additional analyses about the association between the abovementioned subgroups of the environmental

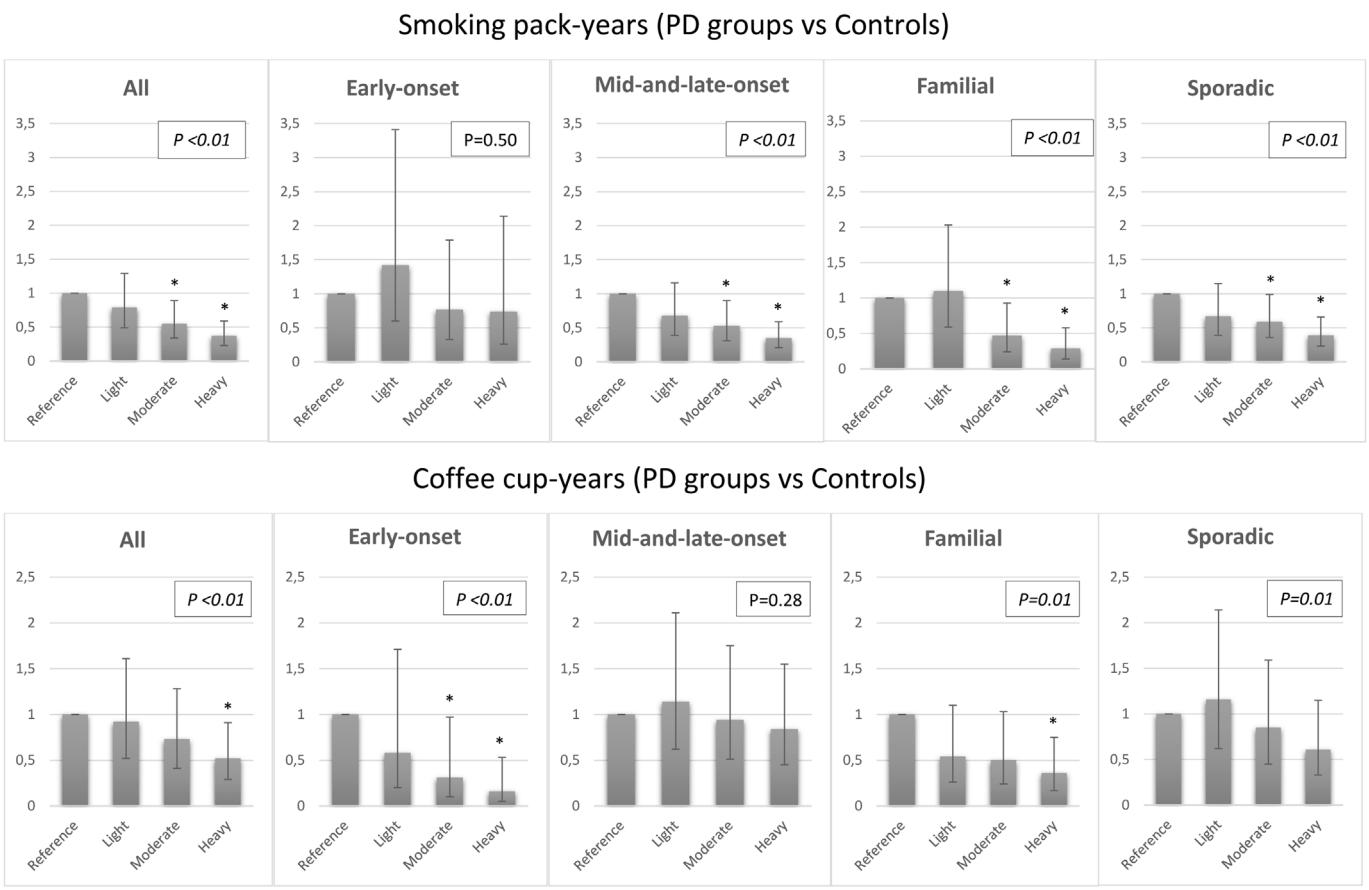

Fig. 1. Odds Ratios, positive and negative error values (calculated from 95% Confidence Intervals) and P for linear relationship estimated for the association between environmental factors and PD overall, as well as PD subtypes (early-onset PD, mid-and-late onset PD, familial PD, sporadic PD), grouped by environmental factor (smoking and coffee consumption). Statistically significant associations for P for linear relationships are in italics. Cls: Controls.
*: Statistically significant associations.

factors and different PD subtypes (early-onset PD patients versus controls, mid-and-late onset PD patients versus controls, familial PD patients versus controls, and sporadic PD patients versus controls). For all of these analyses, we calculated odds ratios (ORs) and their 95% confidence intervals (CI) for each subgroup of the environmental factors, via binary logistic regression models, using simultaneously age, gender and each one of the other environmental factors as covariates.

To investigate the presence of linear relationships we included each exposure category (smoking pack-years group, coffee cup-years group) as a continuous variable in the regression model, and we estimated the p for linear relationship for each of them.

3. Results

3.1. Basic demographic, lifestyle and clinical characteristics of all PD patients, PD patients subtypes and controls

A total of 1042 participants were included in our final analysis. At the time of recruitment, the mean age was quite similar between the two groups, with PD patients being approximately 2 years older than controls ($p < 0.01$) (Table 1). There was a male predominance in the PD group (59% vs 41%), and the mean onset age of PD was in the early sixties (62 years of age). About one out of five patients had early-onset PD (19%), and almost one-third of the patients had a positive family history of PD (31%) (Tables 1 and 2). Due to missing data about the onset age of 11 PD patients, the total number of early-onset PD patients was 129 and of mid-and-late-onset PD patients 546. Furthermore, the total number of familial PD patients was 207 and of sporadic PD patients 468, since data about family history was missing for another 11 PD cases. There was an overlap between the early-onset and familial group (n = 50, 7.4% of all PD patients), as well as between the mid-and-late-onset and sporadic group (n = 382, 56.6% of all PD patients).

Data about smoking pack-years, coffee cup-years and pesticide exposure were available for the vast majority of our subjects: 309 (87%), 305 (86%) and 346 (97%) controls respectively, and for 581 (85%), 550 (80%) and 672 (98%) PD patients respectively. Compared to those with available smoking information (n = 890, 85%), participants with missing smoking information were more commonly women (63% vs. 37%%; $p < 0.01$), while there was no difference in terms of age ($p = 0.66$). Subjects with available (n = 855, 82%) vs those with missing information regarding coffee drinking differed neither in terms of sex ($p = 0.17$), nor in terms of age ($p = 0.22$). Similarly compared to participants with available pesticide exposure information (n = 1018, 98%), those with missing such information were of similar age ($p = 0.87$) and had similar sex distribution ($p = 1$).

Smoking duration and intensity was lower in the PD group as compared to controls ($P < 0.01$ and $P = 0.03$ respectively). The intensity of coffee consumption was lower in the PD group ($P < 0.01$) as compared to controls, whereas there were no significant associations between the duration of coffee intake or pesticide exposure and PD development ($P = 0.65$ and $P = 0.23$ respectively).

Most PD patients did not report a positive family history (n = 468, 69%) and developed mid-and-late-onset PD (n = 546, 81%). At the time of the recruitment, the mean age of the mid-and-late-onset PD subtype was about twenty years higher than the mean age of the early-onset PD subtype (72 vs 51 years of age, $P < 0.01$) (Table 2). The mean age was quite similar between familial and sporadic PD subtypes, with familial PD patients being approximately one and a half year younger

Table 1
Demographic, Lifestyle and Clinical Characteristics of PD patients and controls, Hellenic Biobank of PD, Greece, 2006–2017. SD: Standard Deviation.

Demographic, Lifestyle or Clinical Characteristic	Controls (n = 356)	PD patients (n = 686)
Age		
Mean (SD)	66.26 (9.952)	68.33 (11.936)
Median	66	70.50
Range	53	68
Gender		
Male	131 (36.80%)	407 (59.33%)
Female	225 (63.20%)	279 (40.67%)
Race		
Caucasian	355 (99.72%)	640 (99.69%)
Other	1 (0.28%)	2 (0.31%)
Ethnicity		
Greek	340 (97.98%)	643 (95.12%)
Other	7 (2.02%)	33 (4.88%)
Environmental factors		
Smoking	175 (55.56%)	281 (47.71%)
Smoking years (Mean, SD)	18.05 (19.542)	12.84 (17.061)
Cigarettes per day (Mean, SD)	11.64 (15.196)	9.87 (15.007)
Smoking pack-years (Mean, SD)	19.71 (28.674)	14.41 (26.541)
0	140 (45.31%)	307 (52.84%)
0.01–14.99	45 (14.56%)	92 (15.83%)
15–39.99	59 (19.09%)	100 (17.21%)
≥40	65 (21.04%)	82 (14.11%)
Coffee consumption	289 (88.92%)	498 (86.31%)
Coffee years (Mean, SD)	34.13 (17.291)	33.50 (18.860)
Coffee cups per day (Mean, SD)	1.58 (0.948)	1.37 (0.963)
Coffee cup-years (Mean, SD)	58.67 (42.311)	53.22 (43.251)
0	29 (9.5%)	76 (13.82%)
0.01–40	86 (28.2%)	176 (32%)
40.01–72.99	82 (26.89%)	156 (28.36%)
≥73	108 (35.41%)	142 (25.82%)
Pesticide Exposure	49 (14.16%)	120 (17.86%)
Pesticide Exposure Years (Mean, SD)	1.90 (6.917)	2.88 (8.965)
Pesticide Exposure Type		
None	297 (86.34%)	552 (83.64%)
Indirect	10 (2.9%)	20 (3%)
Gardening	22 (6.4%)	52 (7.9%)
Occupational	15 (4.4%)	36 (5.5%)
Onset Age of PD		
Mean (SD)	–	62.26 (12.729)
Median	–	65
Range	–	67 (22–89)
Early-onset PD	–	129 (19.11%)
Familial PD	–	207 (30.67%)

than sporadic PD patients (P = 0.16). There was a male predominance in all PD subtypes.

3.2. Environmental factors and PD development

When treated dichotomously, smoking was associated with an about 50% lower risk of PD overall (OR 0.48, 95% CI 0.35–0.67), mid-and-late onset (0.46, 0.32–0.66), familial (0.53, 0.34–0.83) and sporadic PD (0.46, 0.32–0.65) in comparison to controls, adjusted for age, gender and each of the other environmental factors (Table 3). There was a linear dose-response association between smoking pack-years and PD (P for linear relationship < 0.01), as compared to controls (Fig. 1).

When dichotomously treated, coffee consumption was associated with an about 65% lower risk of early-onset PD only in comparison to controls (OR 0.36, 95% CI 0.15–0.90), adjusted for age, gender and each of the other environmental factors. Considering different coffee exposure categories, "heavy" coffee-drinking (≥73 cup-years) was associated with a lower risk not only of early-onset PD (0.16, 0.05–0.53), but also of familial (0.36, 0.17–0.75) and PD overall (0.52, 0.29–0.91) as compared to controls, adjusted also for age, gender and each of the other environmental factors. No associations with mid-and-late-onset or sporadic PD were detected. There was a linear dose-response association between coffee cup-years and PD overall, as well as the subtypes of early-onset, familial and sporadic PD, but not mid-and-late-onset PD versus controls (Fig. 1).

We also ran two supplementary analyses, incorporating all subgroups in a given model (i. controls, early-onset and mid-and-late-onset PD patients and ii. controls, familial and sporadic PD patients), whose results confirmed our abovementioned findings. Furthermore, via a logistic regression model, we found that the observed associations between smoking or coffee and the onset-age of PD were independent of the presence of a family history. We describe these two last additional analyses in the Supplementary Material in the Results section.

We found no significant associations between PD and pesticide exposure, including duration and type/intensity of exposure (Table 3).

4. Discussion

In this Greek cohort, we found that smoking was associated with a lower risk of PD, independently of coffee consumption and pesticide exposure. Interestingly, smoking was associated with a lower risk of mid-and-late onset PD, familial PD, as well as sporadic PD, but not with early-onset PD, as compared to controls. Of all PD subtypes, early onset PD was the only one associated with coffee consumption when treated as a dichotomous variable. Early onset PD was also highly associated with "moderate" and "heavy" coffee drinking. Furthermore, independently of smoking and pesticide exposure, "heavy" coffee-drinking was associated with lower odds of PD overall and of familial PD, in comparison to controls. No associations were found between PD and pesticide exposure in our study.

The mean onset age of PD was approximately 63 years, similar to most epidemiological studies reporting mean onset of PD in the early 60s [9]. It is well recognized that more males than females suffer from PD by a ratio of approximately 2:1 [15]. In our study, about 60% of PD cases were men and 40% were women.

While most studies have indicated that 5–10% of patients have early-onset PD, the proportion of early-onset PD was higher in our sample (almost 20% of all PD cases) [9]. In the same context, whereas approximately 10%–20% of all PD patients report a positive family history in most studies [16], a higher percent (approximately 30%) of PD cases were familial in our study. These discrepancies can be attributed to selection bias, since the largest proportion of our data were derived from the Movement Disorder Clinic of Attikon University Hospital, where undiagnosed patients with earlier onset, atypical phenotype or familial forms of the disease (given the known involvement of the Department in genetic studies) were more likely to visit. Alternatively, there may be a larger proportion of familial PD cases in the Greek population.

4.1. Relationship between smoking and PD subtypes

Most epidemiological studies and their meta-analyses have indicated a reduced risk of PD in smokers [3], highlighting the possible protective role of smoking against PD. A previous study had ascertained a relatively low percentage of smokers amongst PD patients in Greece, however no control group was assessed [17]. In the present report we demonstrate for the first time an inverse relationship between smoking and PD in the Greek population. Importantly, we provided evidence that this relationship holds true for most PD subtypes. We also noted an inverse dose-response association between smoking and each of these PD subtypes.

The estimated risk of PD in non-ever smokers was about twice that of ever-smokers. After separate analyses for early-onset, mid-and-late-onset, familial and sporadic PD, smoking was associated with a reduced PD risk broadly in all these different subtypes versus controls, except for early-onset PD, independently of family history. Importantly, since age and environmental exposure duration could affect our results regarding PD subtypes, these variables were both taken into account in our analyses (adjustments for age, smoking pack-years and coffee cup-years

Table 2
Demographic, Lifestyle and Clinical Characteristics of PD subtypes (early-onset PD, mid-and-late-onset PD, familial PD, sporadic PD), Hellenic Biobank of PD, Greece, 2006–2017. SD: Standard Deviation.

Demographic, Lifestyle or Clinical Characteristics	Early-onset PD (n = 129)	Mid-and-late-onset PD (n = 546)	Familial PD (n = 207)	Sporadic PD (n = 468)
Age				
Mean (SD)	51.48 (10.794)	72.47 (7.889)	67.12 (12.561)	68.70 (11.681)
Median	51	73	69	71
Range	55	42	63	68
Gender				
Male	73 (56.59%)	328 (60.07%)	118 (57.00%)	284 (60.68%)
Environmental factors				
Smoking	67/114 (58.77%)	211/465 (45.38%)	86/175 (49.14%)	191/406 (47.04%)
Smoking Years (Mean, SD)	12.88 (14.577)	13.01 (17.668)	11.44 (15.627)	13.48 (17.611)
Cigarettes per day (Mean, SD)	9.39 (12.134)	10.14 (15.742)	8.99 (14.183)	10.34 (15.450)
Smoking pack-years (Mean, SD)	11.68 (17.935)	15.316 (28.420)	12.31 (27.142)	15.44 (26.406)
0	47 (41.23%)	253 (55.36%)	89 (51.45%)	214 (53.5%)
0,01–14.99	31 (27.19%)	59 (12.91%)	39 (22.54%)	51 (12.75%)
15–39.99	27 (23.68%)	73 (15.97%)	27 (15.61%)	72 (18%)
≥40	9 (7.9%)	72 (15.75%)	18 (10.41%)	63 (15.75%)
Coffee consumption	96/114 (84.21%)	394/454 (86.78%)	148/178 (83.15%)	342/391 (87.47%)
Coffee years (Mean, SD)	23.04 (14.521)	36.12 (18.867)	31.66 (19.559)	34.10 (18.473)
Coffee cups per day (Mean, SD)	1.45 (1.035)	1.35 (0.946)	1.34 (0.964)	1.38 (0.970)
Coffee cup-years (Mean, SD)	38.36 (28.718)	56.91 (45.604)	51.79 (44.991)	53.53 (42.532)
0	17 (15.6%)	58 (13.33%)	30 (17.86%)	46 (12.3%)
0,01-40	51 (46.79%)	125 (28.74%)	49 (29.17%)	125 (33.42%)
40,01–72.99	28 (25.69%)	125 (28.74%)	45 (26.79%)	108 (28.88%)
≥73	13 (11.93%)	127 (29.2%)	44 (26.19%)	95 (25.40%)
Pesticide Exposure	19/127 (14.96%)	101/535 (18.88%)	35/200 (17.50%)	85/464 (18.32%)
Pesticide Exposure Years (Mean, SD)	1.29 (4.554)	3.32 (9.760)	2.65 (9.073)	3.03 (8.998)
Pesticide Exposure Type				
None	108 (85.71%)	434 (82.82%)	165 (84.18%)	379 (83.11%)
Indirect	9 (7.1%)	11 (2.1%)	6 (3.1%)	14 (3.1%)
Gardening	6 (4.8%)	46 (8.8%)	17 (8.7%)	35 (7.7%)
Occupational	3 (2.4%)	33 (6.3%)	8 (4.1%)	28 (6.1%)
Onset Age of PD				
Mean (SD)	42.04 (6.978)	67.03 (8.327)	60.23 (13.295)	62.97 (12.419)
Median	44	67	62	65
Range	28	38	61	65
Early-onset PD	–	–	50 (24.51%)	79 (17.14%)
Family history of PD	50 (38.76%)	154 (28.73%)	–	–

were made). One reasonable explanation for this finding is the expected stronger genetic component of early-onset PD [18] that could diminish the protective effect of smoking in this subtype, which is more genetically predetermined and less reliant on environmental factors. In this context, we can only speculate of a more significant genetic contribution in these cases, as genetic testing beyond the SNCA and GBA genes was not performed. Another possible explanation is that smoking could actually delay the age of onset of PD development, thus not manifesting as a protective factor in early onset cases. Alternatively, prolonged exposure to smoking at ages before the pathological process of PD has started may be needed in order for its potential protective effects to manifest. Nevertheless, these possible explanations strengthen the hypothesis of a "true" protective role of smoking in PD. Importantly, the consistent negative association of ever smoking to all other PD subtypes indicates that the putative protective effect of smoking spans across all these different forms of the disease. A previous study conducted in United Kingdom demonstrated an inverse relationship between smoking and PD, independently of the age of onset of the disease [19]. However, the number of participants with early-onset PD in this study was particularly small, and no adjustments for coffee or other factors were made. Quite surprisingly, another study showed that ever-smokers with a family history had the highest risk of PD, but this finding was restricted to people older than 75 years of age, and only 6 subjects were in this group [20]. To the best of our knowledge, this is the largest study investigating the relationship between smoking and PD subtypes, taking into account coffee intake and pesticide exposure.

Nonetheless, the increased mortality of smokers due to other causes could result in their possible under-representation amongst PD patients [21]. On the other hand, the inverse association of smoking with PD with older age of onset in our study, diminishes this possibility. Overall, given the case-control design of our study, we cannot establish causality.

Regarding potential underlying molecular mechanisms, nicotine may act neuroprotectively in PD via modifying the enzymatic activity of monoamine oxidase B (MAO-B), regulating mitochondrial complex I, stimulating the presynaptic release of dopamine, as well as being involved in apoptotic and necrotic pathways [22]. Importantly, some genetic factors, such as the single nucleotide polymorphisms (SNPs) rs356219 and rs356220 in the *SNCA* gene [23] and A/G polymorphism in the MAO-B intron 13 have been demonstrated to modify the PD risk in smokers [24]. In our study, we excluded patients with the A53T mutation in *SNCA* or *GBA* mutations in order to minimize possible genetic bias. Nevertheless, *SNCA* polymorphisms were not assessed.

4.2. Relationship between coffee intake and PD subtypes

Many epidemiological studies have repeatedly reported an inverse link between coffee intake and PD risk, although this association is not as strong and consistent as that of smoking [2]. At a molecular level, experimental evidence suggests that caffeine and its metabolites may inhibit dopaminergic degeneration by acting as adenosine A_{2A} receptor antagonists [2].

Coffee consumption was associated with an about 65% reduced odds of early-onset PD, as compared to controls. Further analyses showed that "heavy" coffee-drinking (≥73 cup-years) was also inversely associated with PD overall and familial PD, but not with sporadic or mid-and-late-onset PD versus controls. A dose-response association was also observed in all subgroup analyses, apart from mid-

Table 3
Odds ratios, 95% confidence intervals and p-values for PD (and subtypes thereof) status in relation to exposure to different environmental factors obtained from logistic regression models adjusted for age, gender and each of the other environmental factors. Smoking pack-years categories: Light (0.01–14.99 pack-years), Moderate (15–39.99 pack-years), Heavy (≥40), Coffee cup-years categories: Light (0.01–40 cup-years), Moderate (40.01–72.99 cup-years), Heavy (≥73 cup-years). Statistically significant associations are highlighted in bold. Cls: Controls, OR: Odds Ratio, CI: Confidence Intervals.

Environmental factors								
PD groups (vs CLs)	OR (95% CI)	P-Value	OR (95% CI)	P-Value	Exposure categories	OR (95% CI)	P-Value	P for linear relationship
Smoking								
	Ever-Smoking (No/Yes)		Smoking-pack years		Smoking pack-years categories			
All	**0.48 (0.35–0.67)**	**< 0.01**	**0.99 (0.98–0.99)**	**< 0.01**	No[a]	1		
					Light	0.79 (0.49–1.29)	0.35	
					Moderate	**0.55 (0.34–0.89)**	**0.02**	**< 0.01**
					Heavy	**0.37 (0.23–0.59)**	**< 0.01**	
Early-onset	0.75 (0.41–1.36)	0.34	0.99 (0.98–1.01)	0.19	No[a]	1		
					Light	1.42 (0.60–3.41)	0.43	
					Moderate	0.77 (0.33–1.79)	0.55	0.50
					Heavy	0.74 (0.26–2.14)	0.58	
Mid-and-late- onset	**0.46 (0.32–0.66)**	**< 0.01**	**0.99 (0.98–0.99)**	**< 0.01**	No[a]	1		
					Light	0.68 (0.39–1.16)	0.16	
					Moderate	**0.53 (0.31–0.90)**	**0.02**	**< 0.01**
					Heavy	**0.35 (0.21–0.59)**	**< 0.01**	
Familial	**0.53 (0.34–0.83)**	**0.01**	**0.98 (0.97–0.99)**	**< 0.01**	No[a]	1		
					Light	1.10 (0.59–2.03)	0.77	
					Moderate	**0.47 (0.24–0.93)**	**0.03**	**< 0.01**
					Heavy	**0.29 (0.14–0.58)**	**< 0.01**	
Sporadic	**0.46 (0.32–0.65)**	**< 0.01**	**0.99 (0.98–0.99)**	**< 0.01**	No[a]	1		
					Light	0.67 (0.39–1.15)	0.15	
					Moderate	**0.59 (0.36–0.99)**	**0.05**	**< 0.01**
					Heavy	**0.39 (0.23–0.66)**	**< 0.01**	
Coffee consumption								
	Ever-coffee drinking (No/Yes)		**Coffee cup-years**		**Coffee-cup years categories**			
All	0.84 (0.53–1.31)	0.44	1.00 (0.99–1.00)	0.11	No[a]	1		
					Light	0.92 (0.52–1.61)	0.76	
					Moderate	0.73 (0.41–1.28)	0.27	**< 0.01**
					Heavy	**0.52 (0.29–0.91)**	**0.02**	
Early-onset	**0.36 (0.15–0.90)**	**0.03**	**0.99 (0.98–1.00)**	**0.01**	No[a]	1		
					Light	0.58 (0.20–1.71)	0.33	
					Moderate	**0.31 (0.10–0.97)**	**0.04**	**< 0.01**
					Heavy	**0.16 (0.05–0.53)**	**< 0.01**	
Mid-and-late- onset	1.15 (0.70–1.90)	0.58	1.00 (0.99–1.00)	0.90	No[a]	1		
					Light	1.14 (0.62–2.11)	0.67	
					Moderate	0.94 (0.51–1.75)	0.85	0.28
					Heavy	0.84 (0.45–1.55)	0.57	
Familial	0.60 (0.34–1.05)	0.08	1.00 (0.99–1.00)	0.11	No[a]	1		
					Light	0.54 (0.26–1.10)	0.09	
					Moderate	0.50 (0.24–1.03)	0.06	**0.01**
					Heavy	**0.36 (0.17–0.75)**	**0.01**	
Sporadic	0.95 (0.58–1.57)	0.85	1.00 (0.99–1.00)	0.231	No[a]	1		
					Light	1.16 (0.62–2.14)	0.65	
					Moderate	0.85 (0.45–1.59)	0.61	**0.01**
					Heavy	0.61 (0.33–1.15)	0.13	
Pesticide exposure								
	Pesticide exposure (No/Yes)		**Pesticide exposure years**		**Pesticide exposure type**			
All	1.00 (0.67–1.51)	0.98	1.02 (0.99–1.06)	0.25	No[a]	1		
					Indirect	0.86 (0.30–2.50)	0.78	
					Gardening	0.65 (0.28–1.53)	0.32	
					Occupational	0.70 (0.25–1.94)	0.49	
Early-onset	0.90 (0.40–2.05)	0.81	1.01 (0.93–1.09)	0.90	No[a]	1		
					Indirect	1.96 (0.38–10.23)	0.43	
					Gardening	0.67 (0.11–4.07)	0.66	
					Occupational	0.39 (0.05–2.98)	0.36	
Mid-and-late- onset	1.04 (0.67–1.60)	0.87	1.02 (0.98–1.05)	0.37	No[a]	1		
					Indirect	0.52 (0.14–1.88)	0.32	
					Gardening	0.75 (0.30–1.83)	0.52	
					Occupational	0.85 (0.28–2.58)	0.78	
Familial	1.11 (0.65–1.90)	0.70	0.99 (0.94–1.04)	0.68	No[a]	1		
					Indirect	1.90 (0.53–6.82)	0.32	
					Gardening	1.23 (0.40–3.79)	0.72	
					Occupational	0.30 (0.04–2.10)	0.23	

(*continued on next page*)

Table 3 (*continued*)

Environmental factors								
PD groups (vs CLs)	OR (95% CI)	P-Value	OR (95% CI)	P-Value	Exposure categories	OR (95% CI)	P-Value	P for linear relationship
Smoking								
	Ever-Smoking (No/Yes)		Smoking-pack years		Smoking pack-years categories			
Sporadic	0.95 (0.61–1.48)	0.81	1.04 (1.00–1.08)	0.08	No[a]	1		
					Indirect	0.48 (0.14–1.69)	0.25	
					Gardening	0.43 (0.17–1.14)	0.09	
					Occupational	0.73 (0.26–2.06)	0.55	

[a] Reference Group.

and-late-onset PD. Since the genetic burden of early-onset and familial PD is considered stronger, these interesting results raise the suggestion that the protective role of coffee in PD may at least depend on its interaction with specific genetic factors, highlighting the importance of possible synergistic effects at least in the Greek population. A previous study showed that caffeine takers with higher genetic susceptibility to PD (*LRRK2* R1628P carriers) had decreased risk of PD, in comparison to non-carriers [25]. Given the fact that a previous study using HBPD data showed that the occurrence of mutations in *Parkin*, *PINK1*, *DJ-1* and *LRRK2* was especially rare in Greece [12], as well as the fact that patients with known *SNCA* or *GBA* mutations were excluded from our analysis, other unknown genetic risk factors could be conceivably associated. In this context, some case-control studies have demonstrated that specific polymorphisms in the *ADORA2A* gene, encoding the adenosine A2A receptor, the *GRIN2A* gene, encoding GluN2A protein that is a subunit of a subset of NMDA receptors, as well as in the *CYP1A2* gene, encoding Cytochrome P450 1A2 that metabolizes caffeine, may affect PD risk [26]. Neither genetic nor environmental factors can separately completely explain PD pathogenesis, as indicated by Genome-Wide Association Studies (GWAS), as well as their meta-analysis [27]. On the contrary, a complex interaction between the genetic profile of each individual and environmental factors may actually underlie PD development in most cases.

It is possible that the reduction in cigarette smoking or coffee consumption could reflect an attempt of PD patients with tremor to reduce nicotine or/coffee-associated tremor, as has been already proposed by some researchers [28]. In this context, we found that there was no significant association between the presence of tremor in PD and smoking or coffee (Supplementary Material, Results section), arguing against this possibility.

4.3. Relationship between pesticide exposure and PD subtypes

Although many epidemiological studies have identified that exposure to pesticides may increase PD risk [29], we found no relevant associations, even after the subcategorization of the self-evaluated exposure intensity (indirect, gardening and occupational). Assessment of pesticide exposure was based on self-evaluation and not on objective quantitative measurements in our study. Furthermore, some possible confounding factors, involving rural living and well-water consumption that have been also reported to increase PD risk [29] were not taken into account in our study. Moreover, no discrimination between different types of pesticides was done, so potential associations between specific types of pesticides and PD may have been missed. An additional intriguing possibility is that pesticide exposure may increase the risk of PD only in select subpopulations with a specific genetic substrate for PD. Along these lines, we have reported a marginally significant higher exposure to pesticides in *GBA* mutation carriers compared to matched genetically unidentified PD patients from our cohort [30]. However, the size of the sample was quite small. Clearly, more work is needed along these lines, with a larger number of subjects with genetic PD, in order to investigate potential associations between specific genetic PD forms and exposure to environmental factors.

4.4. Limitations and strengths of our study

Our study has certain limitations. There could be selection bias, since the largest proportion of our data are derived from Attikon University Hospital, where undiagnosed patients with atypical characteristics are more likely to visit. There is potential recall bias due to the self-assessment of the exposure, especially in PD patients, who are more likely to have cognitive decline. Moreover, no discrimination between different types of pesticides was done, so potential relevant associations may have been missed. Additionally, although HBPD does contain data about the existence of tremor, bradykinesia, rigidity and various non-motor features, there is no categorization into tremor-dominant, akinetic-rigid or other PD phenotypic subtypes. As a result, we were not able to make such classifications in our study. Furthermore, since we based our analysis on a retrospective case-control study design, we cannot establish causation regarding the relationships we found between environmental exposures and PD development and reverse causality cannot be excluded.

At the same time, our study has several strengths. To the best of our knowledge, this is the largest clinical study aiming to investigate the association between environmental exposures and PD, and in particular major PD subtypes, in the Greek population. The quantitative data on environmental exposures largely confirm dose-dependent relationships for both coffee drinking and smoking. Moreover, we took into account that coffee, smoking, or pesticide exposure could possibly act as confounding factors for each other. We used the database of the HBPD, that included careful and detailed collection and documentation of demographic, clinical and exposure information, therefore increasing used data quality. All PD patients and controls were examined by neurologists specialized in movement disorders. The exclusion of patients with the A53T mutation in the *SNCA* gene or *GBA* mutations limits possible bias from known common genetic influences, leading to safer conclusions. Finally, the largest proportion of our participants came from various geographical areas in Greece, leading to geographic variability and allowing the relative generalizability of our results, at least within the Greek population.

5. Conclusions

This is the largest and, to our knowledge, the only clinical study aiming to investigate the association between environmental exposures and PD, in particular different PD subtypes, in the Greek population. In the HBPD population, smoking was associated with a reduced risk of all PD subtypes apart from early-onset PD, in comparison to controls. Early-onset PD was strongly associated with reduced coffee intake, using both qualitative and quantitative assessments. “Heavy” coffee-drinking was inversely associated with PD overall and familial PD versus controls, whereas a dose-response was observed in all PD

subgroups, apart from mid-and-late-onset PD. These results highlight the importance of potential gene-environment interactions for the protective role of coffee in PD. Towards this direction, additional analyses of exposure to environmental factors and their interplay with genetic factors are needed in order to advance the understanding of PD pathogenesis.

Authors' roles

Efthalia Angelopoulou: 1,2,3,4,5.
Maria Bozi: 2,3,5.
Athina-Maria Simitsi: 2,3,5.
Christos Koros: 2,3,5.
Roubina Antonelou: 2,3,5.
Nikolaos Papagiannakis: 2,3,5.
Matina Maniati: 2,3,5.
Dafni Poula: 2,3,5.
Maria Stamelou: 2,3,5.
Demetrios K. Vassilatis: 1,2,3,5.
Ioannis Michalopoulos: 1,2,3,5.
Styliani Geronikolou: 1,3,5.
Nikolaos Skarmeas: 3,5.
Leonidas Stefanis: 1,2,3,5.

1: conception and design of the study, 2: acquisition of data, 3: analysis and interpretation of data, 4: drafting the manuscript, 5: review and critique of the manuscript for important intellectual content.

All authors have approved the final version of the manuscript.

Conflicts of interest

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.08.013.

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