Relationship between risk and protective factors and clinical features of Parkinson's disease

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ABSTRACT

Background: Non-genetic risk factors play a relevant role in Parkinson's disease (PD) development but the relationship between these factors and PD clinical features is unknown.

Objective: The aim of the present multicenter study was to investigate possible relationship between risk factors and clinical motor and non-motor features in a large sample of PD patients.

Methods: Six hundred ninety-four patients with PD participated. Patients underwent a clinical evaluation assessing motor symptom and motor complication as well as non-motor symptom severity. Information regarding pharmacological treatment was also collected. Risk and protective factors were previously identified in the present population and included coffee consumption, cigarette smoking, and physical activity as protective factors and a family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia as risk factors. Multiple regression models were used to investigate the relationship between risk factors and clinical variables.

Results: Coffee consumption predicted older age at onset (B: 0.527; CI: 0.195; 0.858) and milder motor symptom severity (B :-1.383; CI: -2.646; -0.121). Non-motor symptom severity was more severe in patients with dyspepsia before PD (B: 13.601; CI 5.019; 22.182) and milder in patients who performed physical activity before PD (B: -11.355; CI:-16.443; -6.266). We found no relationship between risk factors and motor complications, motor subtype and pharmacological treatment.

Conclusions: Risk and protective factors of PD development may influence PD clinical features. This finding may represent the first step in the development of new preventive approaches able to delay disease onset and mitigate the extent of clinical manifestations.

INTRODUCTION

Recent evidence has shown that genetic risk factors may influence Parkinson's disease (PD) clinical features, including age at onset, motor phenotype, and motor and non-motor symptom severity [1,2]. However, a number of studies have also demonstrated the importance of several non-genetic risk and protective factors of PD development [3,4].

In a recent paper, we retrospectively assessed 694 patients and 640 healthy controls to explore the potential risk or protective role of 31 non-genetic endogenous and exogenous factors that had been previously suggested to increase or decrease PD risk [4]. Using a logistic regression model, we observed that a family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia were the most relevant independent risk factors for PD development [4]. Conversely, coffee consumption, cigarette smoking, and physical activity were the main independent protective factors against PD [4]. Despite the importance of the above mentioned non-genetic risk factors in PD development, the relationship between these factors and PD motor and non-motor features is still uninvestigated and no studies have explored the role of the risk factor profile in determining clinical manifestations of PD.

In the present paper, we provided a clinical description of the PD patients enrolled in our previous study [4], and explored the relationship between risk/protective factors and PD clinical motor and non-motor features, including age at onset, motor symptom and complication severity, non-motor symptom severity, motor phenotype and pharmacological treatment.

METHODS

A total of 694 patients affected by PD participated in the study. Patients were enrolled from among consecutive outpatients with PD who attended follow-up visits at the movement disorder clinics of six neurological departments. PD was diagnosed by senior neurologists, expert in movement disorders according to standard published criteria [5].

All patients were interviewed and underwent a clinical assessment performed on treatment by a neurologist expert in movement disorders. Age at disease onset was considered as the age when motor symptoms appeared. Disease stage was evaluated using the Hoehn & Yahr (H&Y) scale [6]. Motor symptoms were evaluated by the International Parkinson and Movement Disorder Society-sponsored Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III [7]. Motor complications were evaluated by the MDS-UPDRS part IV [7]. Non-motor symptom manifestations were evaluated by the Non-Motor Symptoms Scale (NMSS) for PD [8]. This scale assesses different non-motor domains, including cardiovascular symptoms, sleep disturbances, autonomic dysfunction, cognitive impairment, neuropsychiatric dysfunction, genitourinary dysfunction, gastrointestinal dysfunction, and olfactory function [8].
Levodopa equivalent daily doses (LEDDs) was calculated for each patient.

To investigate the relationship between motor subtype and risk factor profile, we identified clinical motor subtypes using the method described by Stebbins et al., which is based on MDS-UPDRS parts II and III [9]. Using this method, we classified PD patients as having a tremor dominant (TD), postural instability/gait disorder (PIGD), or intermediate subtype. As proposed by Stebbins et al., we calculated the TD/PIGD score ratio for each patient by dividing the tremor score by the PIGD score. Patients with a ratio lower than 0.90 were classified as having a PIGD subtype, while patients with a ratio higher than 1.15 were classified as having a TD subtype. Patients with a ratio between 0.90 and 1.15 were classified as having an intermediate subtype.
Risk and protective factors of PD identified in the previous study were assessed using a semi-structured questionnaire [4]. The questionnaire was administered in person by one medical interviewer at each center. The questionnaire assessed the presence of possible risk/protective factors by interviewing patients about the entire life period preceding PD onset. The questionnaire also collected data on demographic features (age, gender, age at motor symptom onset, years of schooling) and family history of parkinsonism (first-degree relatives only). Lifestyle factors investigated included cigarette smoking, coffee consumption, and physical activity. Smoking and

coffee habits were investigated according to a semi-structured questionnaire already used in previous studies on dystonia [10]. Questions on coffee consumption and cigarette smoking referred to the year preceding symptom onset. Subjects were classified as never having smoked or drank (non-smokers/non-drinkers) or as past or current smokers or drinkers. Participants were asked about their physical activity before developing PD. Seventeen physical activities were selected and converted into metabolic equivalents of task according to the Pate model [11]. Given that the level of physical activity may influence the relationship between this factor and PD [12], we considered slight vs moderate/substantial physical activity [4]. Exposure to general anesthesia was also assessed. To investigate the presence of dyspepsia, we asked participants whether they had postprandial fullness, early satiety, epigastric pain, or epigastric burning [4].

We also assessed occupational exposure to pesticides or processes involving oils, metals, solvents, and paints. According to a previous study [13], participants were asked about possible workplace exposure to any chemicals, including solvents, oils, plastics, paints, metals, and pesticides.

The study received approval from the ethical standards committee on human experimentation (Sapienza University of Rome ethics committee n. 4734). Received written informed consent was obtained from all patients participating in the study (consent for research). The data that support the findings of this study are available on reasonable request from the corresponding author.

Statistical analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise specified. Statistical analysis was performed using SPSS software version 25.

In order to assess possible relationship between age at onset and risk factors, we designed a multiple linear regression model with age at onset (expressed in years) as the dependent variable and risk

(family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 1). Current age, sex, and referral center were also included in the model to adjust for the results of multiple regression analysis.

To assess the relationship between motor symptoms severity and risk factors, we designed a multiple linear regression model with MDS-UPDRS part III (score) as the dependent variable and risk (family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 2). Age, sex, referral center, disease duration and LEDDs were also included in the model to adjust for the results of multiple regression analysis.

To assess the relationship between motor complications severity and risk factors, we designed a multiple linear regression model with MDS-UPDRS part IV (score) as the dependent variable and risk (family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 3). Age, sex, referral center, disease duration and LEDDs were also included in the model to adjust for the results of multiple regression analysis.

To test possible relationship between non-motor symptoms severity and risk factors, we designed a multiple linear regression model with NMSS score as the dependent variable and risk (family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 4). Age, sex, referral center, disease duration and LEDDs were also included in the model to adjust for the results of multiple regression analysis.

To assess the relationship between antiparkinsonian pharmacological treatment and risk factors, we designed a multiple linear regression model with LEDDs as the dependent variable and risk (family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective

factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 5). Age, sex, referral center and disease duration were also included in the model to adjust for the results of multiple regression analysis.

Finally, to test the test the relationship between motor subtype and risk factors, we designed a logistic regression model with motor subtype (tremor dominant=1; non-tremor dominant=0) as the dependent variable and risk (family history of PD, dyspepsia, and exposure to toxic agents and general anesthesia) and protective factors (coffee consumption, cigarette smoking, physical activity factors) as independent variables (Model 6). Age, sex, referral center, disease duration and LEDDs were also included in the model to adjust for the results of multiple regression analysis.

In all models dummy variables were used when dichotomus categorical variables were involved in the analysis.

Fifty-nine cases containing missing information were excluded from analysis because clinical information was incomplete.

RESULTS

A total of 694 PD patients met eligibility criteria during the study period and agreed to take part in the study. Mean age at symptoms onset was 60.7 (SD 9.1) years and mean disease duration was 7.2 (SD 6.3) years. Demographic and clinical features of PD patients are reported in Table 1.

Multiple linear regression analysis

The linear regression model assessing possible relationship between risk/protective factors and age at onset showed that only coffee consumption prior to PD onset significantly predicted a higher age at onset (B coefficient 0.527; 95% confidence interval [CI] (0.195;0.858) (Model 1 in Table 2).

The linear regression model testing possible relationships between risk/protective factors and motor symptom severity showed that PD patients who reported coffee consumption before disease onset had a milder motor symptom severity (B :-1.383; CI: -2.646; -0.121) (Model 2 in Table 2). Conversely, no relationship was found between risk/protective factors of PD and motor complications (Model 3 in Table 2).

The linear regression model assessing possible relationship between risk/protective factors and non-motor symptom severity showed a significant relationship between dyspepsia and more severe non-motor severity (B: 13.601; CI 5.019; 22.182) (Model 4 in Table 2). Since the relationship we observed between non-motor symptom severity and dyspepsia could have been influenced by the gastrointestinal domain of the NMSS, we performed a sensitivity analysis by re-testing the relationship between dyspepsia and NMSS score, excluding the gastrointestinal domain score. We confirmed that dyspepsia was significantly associated with non-motor symptom severity even when excluding gastrointestinal dysfunction (B: 12.43; 95% CI: 4.29; 20.56). Conversely, we observed that physical activity before PD onset significantly predicted milder non-motor symptom severity (B: -11.355; CI:-16.443; -6.266) (Model 4 in Table 2).

We observed no relationship of risk/protective factor of PD with LEDDs (Model 5 in Table 2) and with motor subtype (Model 6 in Table 2).

Finally, we found that cigarette smoking and exposure to toxic agents or general anesthesia before disease onset were not related to PD clinical features.

DISCUSSION

The novelty of the present paper relies on the investigation of possible relationship between well recognized non-genetic risk/protective factors and motor and non-motor clinical features of a large sample of 694 patients with PD. We observed that coffee consumption predicted an older age at

onset and milder motor symptom severity. In particular, the beta score indicated that coffee drinkers will have a 0.5-year delay in age of PD onset compared with non-drinkers, and a reduction of 1.3 points in the UPDRS-II score. Likewise, non-motor symptom severity was higher in patients with dyspepsia before PD onset (dyspeptic subjects have an increase of 13 points in the NMSS score as compared with non dyspeptic subjects) and milder in patients who performed physical activity before PD onset (subjects who performed physical activity have a decrease of 11 points in the NMSS score as compared with subjects who did not). We did not find any association between risk/protective factors and motor complications, motor subtype and LEDDs.

In order to exclude methodological biases that could affect our findings, we took several precautions. First, the examiner who performed the clinical evaluation was not informed about the number and type of risk/protective factor which was associated to PD onset in each patient. This allowed us to avoid possible experimental biases arising from participant expectations, observer effects on participants, and observer bias. Second, given the multicenter design of the study, a possible methodological issue could have been represented by the inter-individual variability of examiners. To minimize this bias, all examiners underwent the same training on data collection and clinical evaluation. Third, since motor and non-motor symptom severity can be influenced by several demographic (age, sex, and referral center) and clinical (disease duration and LEDDs) parameters, we adjusted our multiple regression models for these demographic and clinical parameters.

In the present study, we observed that coffee consumption predicted an older age at onset and milder motor symptom severity, as measured by MDS-UPDRS part III score. The protective effect of caffeine on PD development has been consistently reported [4, 14, 15], and recent longitudinal evidence suggests that caffeine is able to slow PD clinical progression [16]. Caffeine (1,3,7-trimethylxanthin) is a natural alkaloid and is an adenosine receptor antagonist [17]. Experimental models of PD have clearly demonstrated that the neuroprotective effect of caffeine is mediated by the blockade of adenosine A2A receptors predominately expressed in the basal ganglia and by the

consequent modulation of adenosine-dopamine interaction [18,19].In addition, caffeine increases dopamine levels by inhibiting the activity of monoamine oxidase-B [19], and protects dopaminergic neurons by activating anti-oxidative signaling pathways, such as nuclear factor erythroid 2-related factor 2 (Nrf2)-Keap1 and peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1) [19]. Evidence suggests that dopaminergic-loss-related basal ganglia dysfunction is the pathological substrate of motor symptoms in PD [20], and PD clinical diagnosis is currently based on the presence of motor symptoms [5]. Therefore, the present observation that exposure to caffeine before PD development predicted an older age at onset and milder motor symptom severity may be explained by the neuroprotective effect that caffeine exerts on dopaminergic degeneration and basal ganglia dysfunction.

Regarding the relationship between risk factors and non-motor symptom severity, we found that NMSS score was lower in patients who performed physical activity before PD onset and higher in pazients with a clinical history of dyspepsia. From a pathophysiological [21], biological [22], and pathological perspective [23], PD non-motor symptoms reflect the multisystem involvement of dopaminergic (striatal and extrastriatal) and non-dopaminergic (acetylcholine, noradrenaline, serotonin, hypocretin, and substance P) pathways. Animal models have suggested that physical activity likely reduces PD risk through the exertion of neuroprotective and restorative effects on the nigrostriatal dopaminergic system by increasing glial-cell-line-derived neurotrophic factor (GDNF) [24] and brain development neuroprotective factor (BDNF) [25], improving glutamate neurotransmission [26], and increasing the availability of striatal dopaminergic neurons [26]. At the same time, physical exercise may modulate non-dopaminergic monoamine neurotransmitters, like noradrenergic and serotonergic systems [27], and prevent the cholinergic dysfunction that represents the biological substrate of neurodegenerative dementia [28]. Therefore, motor exercise may modulate not only dopaminergic system but also serotoninergic, noradrenergic, glutammatergic and cholinergic neurotransmission. It is, therefoe, biologically plausible that

physical activity performed before PD development may exert a multisystem neuroprotective effect on the neural structures and circuits involved in PD non-motor symptoms.

We also found a relationship between a clinical history of dyspepsia and non-motor symptom severity. Since the NMSS includes a gastrointestinal dysfunction domain, a possible explanation for this finding could be the increased severity of gastrointestinal symptoms in patients with dyspepsia. However, we performed a sensitivity analysis by excluding gastrointestinal domain items from the NMSS global score and we confirmed the significant association between dyspepsia and non-motor symptom severity. Dyspepsia may reflect gut inflammation, which plays a key role in the pathophysiology of PD according to a growing body of evidence [29]. The presence of dyspepsia as a risk factor for PD might support the hypothesis that PD-related pathophysiological processes start at the gut level, with subsequent brain involvement [29]. According to this hypothesis, alpha-synucleinopathy may ascend retrogradely via the vagus nerve to neurons of the dorsal motor nucleus in the brainstem to reach the substantia nigra via the nigro-vagal pathway [29]. This type of alpha-synucleinopathy propagation may be related to multi-pathway involvement with consequent appearance of non-motor symptoms.

In the present study, no relationship between PD clinical features and cigarette smoking was observed. Cigarette smoking has been consistently reported as a protective factor for PD development and it has been speculated that nicotine may exert its protective effect by acting on nicotine receptors at central nervous system level [30], or alternatively by modifying microbiome at gut level [3, 4]. Cigarette smoking is a recognized protective factor also of other neurodegenerative conditions, such as Alzheimer's disease [28]. It is, therefore, possible that cigarette smoking exerts a non disease-specific neuroprotective effect that intervenes in the first stages of neurodegeneration.

Similarly, we found that family history of PD, exposure to toxic agents and to general anaesthesia did not predict motor features of PD patients, thus suggesting that these factors are relevant in the pathogenetic mechanisms that intervene in causing PD but do not play a major role in influencing

the clinical features of PD. Alternatively, these factors may play a role in modulating PD clinical features at early stages of the disease with this role being less evident as disease progresses. Future studies investigating de novo PD patients may clarify this point.

Furthermore, we found no relationship between risk/protective factors of PD and motor complications and pharmacological treatment dosage. Given that motor complications and pharmacological treatment dosage are strongly influenced by a number of factors that intervene during disease course rather than prior to disease onset it is plausible that risk factors are not relevant in modulating these clinical variables.

In our study, we investigated the possible relationship between risk/protective factors of PD and parkinsonian clinical features. Although we investigated exposure to risk and protective factors in the period that preceded PD development, it is possible that the same factors may still be present after diagnosis and may influence disease progression. While this consideration is not applicable to the relationship between caffeine and age at disease onset, it is conceivable for the protective effects exerted by caffeine and physical activity on motor and non-motor manifestation severity, respectively. This hypothesis is in line with a previous prospective study that reported an inverse relationship between lifelong exposure to these two factors and PD clinical progression [16]. Therefore, the next step should be to design longitudinal studies assessing the neuroprotective power of caffeine and physical activity on prodromal, early, and moderate/advanced phases of PD.

The present study has some limitations. The major limitation is due to the cross-sectional nature of the study. The relationships between variables that emerged from this study should thus be interpreted with caution since follow-up longitudinal investigations are needed to confirm them. In addition, the relatively long disease duration of our patients might have blurred many phenotypic relationships evident at an earlier disease stage. Finally, we did not include in our data collection some relevant PD clinical variables, like dementia and falls. However, it is important to point out

that our study included a large, consecutively enrolled, multicenter population, which was as representative as possible of the natural history of the disease.

In conclusion, our novel findings suggest that risk and protective factors of PD development are associated with clinical features of PD patients, including age at onset and motor and non-motor symptom severity. The observation that non-genetic risk and protective factors may be associated with PD phenotypic expression may represent the first step in developing new preventive and therapeutic approaches able to delay disease onset or mitigate the extent of clinical manifestations. In this regard, physical activity and caffeine seem to be promising candidates for future preventive and therapeutic approaches in PD patients.

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Financial disclosure/conflict of interest concerning the research related to the manuscript

None of the authors have any potential conflicts of interest to disclose related to the manuscript.

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Data availability statement: The data that support the findings of this study are available on reasonable request from the corresponding author.

Authors contribution: DB designed the study, collected and analysed data, drafted the paper. RP designed the study, collected and analysed data. AF collected data and analysed data. MC collected data and analysed data. SP collected data and analysed data. MDL collected data and analysed data. NM collected data and analysed data. FM collected data and analysed data. CD collected data and analysed data. TE collected data and analysed data. AN participated in writing and revision of the manuscript. MZ participated in writing and revision of the manuscript. PS collected data and analysed data. MB participated in writing and revision of the manuscript. GF participated in the conceptualization, formal analysis, writing and revision of the manuscript. MT participated in the conceptualization, writing and revision of the manuscript. AB designed the study and participated in the conceptualization, writing and revision of the manuscript. GD designed the study and participated in the conceptualization, writing and revision of the manuscript.

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