BRIEF REPORTS

Age at Onset of LRRK2 p.Gly2019Ser Is Related to Environmental and Lifestyle Factors

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Abstract: Objectives: The effect of environmental and lifestyle factors on patients with LRRK2 (leucine-rich repeat kinase 2) p.Gly2019Ser (LRRK2+/PD+) compared to idiopathic PD (iPD) has yet to be thoroughly investigated.

Methods: In a homogeneous Tunisian Arab Berber population, we recruited 200 idiopathic PD and 199 LRRK2 p.Gly2019Ser mutation carriers, of whom 142 had PD (LRRK2+/PD) and 57 were unaffected (LRRK2+/PD°). Case report form (CRF) questionnaires (motor and non-motor symptoms) including the Geoparkinson Questionnaire were used to assess environmental and lifestyle factors.

Results: In LRRK2+/PD°, tobacco use was significantly associated with a later median age at onset (AAO). The median AAO was 60 years (interquartile range = 52–67.25) for tobacco users, compared to 52 years (interquartile range = 45.25–61) for non-users (P = 0.0042 at adjusted α = 0.025). Additionally, we observed an independent but additive effect of black tea consumption and tobacco use.

Conclusions: Our data suggest that tobacco and black tea have a protective effect on age at onset in LRRK2+/PD°. © 2020 The Authors. Movement Disorders published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: LRRK2; environment; age at onset; smoking; black tea; modifiers; penetrance

Parkinson’s disease (PD) is the fastest growing neurological disease and the second most common neurodegenerative disorder characterized clinically by motor dysfunction and currently affecting over 7 million patients worldwide.1,2 Currently, monogenic forms and strong genetic risk factors explain ~10% of PD.3 Mutations in the LRRK2 gene are the most frequent cause of autosomal-dominant PD.4,5 A combination of genetic and/or environmental factors influences PD susceptibility. For example, a meta-analysis has shown that smoking had a robust negative association with PD risk.6 Furthermore, smoking is correlated with a later onset of motor and non-motor symptoms.7 The causal protective relationship of smoking initiation has been supported by a recent Mendelian randomization (MR) study.8 Although a direct causal relationship between PD onset and other lifestyle factors has yet to be established, coffee drinking is equally correlated with reduced risk of PD.6 Slower progression of motor and non-motor symptoms has been shown for coffee consumers in a longitudinal study.9 By contrast, with regard to environmental factors affecting PD, pesticide exposure is associated with an increased risk.6,10 The relationship of environmental and lifestyle factors on age at onset (AAO) of LRRK2 p.Gly2019Ser has not been thoroughly investigated. Herein, we focused on the association of smoking on LRRK2 p.Gly2019Ser mutation carriers. We hypothesize that smoking has a protective effect on LRRK2 p.Gly2019Ser as well. We performed additional exploratory analyses on other lifestyle and environmental exposures.
Methods

Demographic and Participant Examination

From 2002 to 2008, movement disorders specialists at the Institute of Neurology in Tunis recruited participants in Tunisia for a clinical and genetic study of PD (Supplementary Fig. S1). Our study consists of 399 participants: 142 affected mutation carriers (LRRK2+/PD+), 57 unaffected mutation carriers (LRRK2+/PD−), and 200 patients with iPD (Supplementary Table S1). Recruitment details are described in a flow chart (Supplementary Fig. S1). All 399 participants had detailed genealogical, genetic, clinical, and environmental data collected. Clinical examination of the participants was performed by movement disorders specialists, and case report form (CRF) questionnaires were completed for all participants, as previously described. The data were collected via structured interviews in-person on paper CRF forms, followed by the inclusion into an electronic database. All participants completed the Geoparkinson Questionnaire and the Parkinson’s disease risk factor questionnaire (PD RFQ-U) (Supplementary Fig. S2). Details on questionnaires and genetic analysis are described in the Supplementary text. LRRK2 p. Gly2019Ser was genotyped.

Statistical Analysis

GraphPad Prism software (San Diego, CA), JMP (SPSS), and R studio were used for statistical analysis. Non-parametric Mann-Whitney U test was performed to compare AAO, Spearman correlation was used for correlations, and regression model was performed to assess interactions between variables. Cox proportional hazards model was performed to include LRRK2+/PD− (Supplementary text). All reported P values stated are exploratory unless specified. Based on the presence of the “a priori” hypothesis on smoking, differences in AAO were tested for significance between tobacco users and non-users both in LRRK2+/PD+ and iPD patients, and the significance level was adjusted for performing two tests to $\alpha = 0.05/2 = 0.025$.

Results

Tobacco Use and Age at Onset

We first investigated the association between smoking and AAO in LRRK2+/PD+ and iPD. LRRK2+/PD+ who reported use of tobacco had a later AAO (median AAO = 60 years; interquartile range [IQR] = 52–67.25) compared to non-users (median AAO = 52 years; IQR = 45.25–66) ($P = 0.0042$) (Table 1 and Supplementary Table S2). When right-censoring asymptomatic carriers in a Cox proportional hazards model, the effect of tobacco use on AAO was still present ($P = 0.0367$).

Smoking Intensity/Duration and Disease Onset

In addition to just looking at smokers and non-smokers, assessment of whether there is a dosage effect within the smokers can be performed further. Therefore, we performed the analysis on individuals who smoked and did not include non-smokers without information on smoking duration. In this analysis, the number of cigarettes per day showed a correlation with AAO within the smokers can be performed further. Therefore, we performed the analysis on individuals who smoked and did not include non-smokers without information on smoking duration. In this analysis, the number of cigarettes per day showed a correlation with AAO ($r = 0.3734$, $P = 0.0296$) (Fig. 1A). The duration of smoking until disease onset was also correlated with AAO ($r = 0.5895$, $P < 0.0001$) (Fig. 1B). To include asymptomatic carriers, we have right-censored for these

### Table 1. Association of environmental/lifestyle factors with age at onset (AAO) in LRRK2+/PD+ and iPD

| Factor                        | LRRK2+/PD+ | iPD |
|-------------------------------|------------|-----|
| Tobacco use                   | Yes        | No  |
| N                             | 50         | 76  |
| Median AAO (IQR)              | 60 (52.00–67.25) | 52 (45.25–61) |
| Men (%)                       | 45 (90.0)  | 23 (30.3) |
| Black tea consumption         | Yes        | No  |
| N                             | 77         | 45  |
| Median AAO (IQR)              | 58 (48–67) | 52 (46.5–57) |
| Men (%)                       | 41 (53.2)  | 25 (55.6) |
| Pesticide exposure (non-work setting) | Yes | No |
| N                             | 44         | 68  |
| Median AAO (IQR)              | 53.5 (43.25–60) | 55.5 (48–63.75) |
| Men (%)                       | 22 (50)    | 38 (55.9) |

Abbreviations: LRRK2+/PD+, LRRK2 parkinsonism (patients with PD and LRRK2 p.G2019S mutation); iPD, idiopathic PD; IQR, interquartile range; N, number of individuals; NA, not applicable; tobacco use: yes = patient smoked more than 100 cigarettes in their life or used regularly smokeless tobacco, tobacco use: no = patient did not used tobacco; black tea: yes = patient drank black tea once per week for at least 6 months, black tea: no = patient did not drink black tea; pesticide exposure (non-work setting): yes = patient were ever exposed to pesticide in a non-work setting, pesticide exposure (non-work setting): no = patients were never exposed to pesticides in a non-work setting; $P$ value = two-sided exploratory $P$ values from Mann-Whitney U-tests.

*Tested for significance at $\alpha = 0.025$. 

* $P$ values from Mann-Whitney U-tests.
individuals within a Cox proportional hazards model. This again showed that the effect of smoking duration until PD onset on AAO was present in LRRK2+/PD+ \( (P = 0.0001) \) (Supplementary Table S3).

Smoking and Clinical Presentation

To investigate whether tobacco use was associated with other motor or non-motor symptoms, we compared the median clinical MDS UPDRS scores between tobacco users and non-users. Considering only LRRK2+/PD+, the median MDS UPDRS IA, MDS UPDRS IB, and MDS UPDRS IV scores were lower for tobacco users (Supplementary Table S4). In a regression model with disease duration as a covariate, we only observed an effect of tobacco use on MDS UPDRS IA and IB. On the other hand, tobacco use was not associated with changes in the MDS UPDRS II, III and Hoehn & Yahr stage.

Caffeine and Pesticides

Although no difference was observed for median AAO and general consumption of caffeine, black tea drinking alone was observed to be associated with AAO (Supplementary Fig. S3A,B). LRRK2+/PD+ who consumed black tea had a later AAO (median AAO = 58 years; IQR = 48–67) compared to those who did not (median AAO = 52 years; IQR = 46.5–57) \( (\text{Mann-Whitney } U \text{ test } P = 0.0024) \). We observe the same in iPD, with black tea drinkers presenting a later AAO (median AAO = 57 years; IQR = 45.25–66) compared to individuals who did not have black tea (median AAO = 49 years; IQR = 39–60) \( (\text{Mann-Whitney } U \text{ test } P = 0.0034) \).
Caffeinated soda drinkers showed an earlier AAO for LRRK2+/PD+. In the group of patients who consumed caffeinated soda, the median AAO was 49 years (IQR 41.75–57) in contrast to the group of patients that did not consume caffeinated soda (median AAO = 56 years; IQR = 46.5–57) (Mann Whitney U test P = 0.0031). Pesticide exposure in a work or non-work setting did not show association with AAO in LRRK2+/PD+. In iPD, an earlier AAO was observed with exposure to pesticides in a non-work setting (median AAO = 50 years; IQR = 38–62), compared to those that were never exposed (median AAO = 57 years; IQR = 45–65) (Mann Whitney U test P = 0.0367) (Supplementary Fig. S3C,D).

Independent and Joint Effect of Tobacco and Black Tea

After observing the effects of smoking and black tea on AAO, we further explored a joint effect of tobacco use and black tea (Fig. 1C,D). Using a regression model, we found evidence for independent effects of tobacco use and black tea on AAO in LRRK2+/PD+ without evidence for an interaction. In LRRK2+/PD+, tobacco users and black tea drinkers had a later AAO (median AAO = 64 years; IQR = 53–68.5) compared to those who used neither substance (median AAO = 51 years; IQR = 43.5–55.5). In the regression model, the effect of tobacco use on AAO was present in LRRK2+/PD+ (P = 0.00975) and for black tea (P = 0.00335); there is no interaction between tobacco and black tea (P = 0.988). We did not observe an effect of tobacco use on AAO in iPD, only for black tea. In the regression model, the effect of tobacco use and AAO was not present in iPD (P = 0.249), but was present for black tea (P = 0.0080); again, there is no interaction between tobacco and black tea (P = 0.154).

Discussion

Because AAO genetic modifiers have been nominated for LRRK2+/PD+, effect sizes have been small and ethnicity/populations seem to play a role.11,15-18 This study establishes a connection between environmental and lifestyle factors that adds to the complexity in LRRK2+/PD+.

A correlation between smoking and a reduced risk has been previously reported,5,19,20 and a causal protective relationship between smoking initiation (and other risk-taking behaviors) and PD was supported by a MR study.8 Logically, we hypothesized a protective influence of tobacco use on LRRK2+/PD+. We show that tobacco use is associated with later AAO in patients carrying the p.Gly2019Ser mutation, and the intensity and duration of smoking is correlated with AAO in these individuals. Additionally, we observed an effect of tobacco use exclusively on non-motor symptoms but not for motor symptoms after adjustment for disease duration. It is also important to note that there is a much higher ratio of men in the group of tobacco users (90%) in comparison to non-users (30.3%) (Table 1), reflecting cultural preference in Tunisia.21 In this case, smoking effects on AAO could be influenced by gender-specific differences (ie, hormonal or genetic). An interesting joint, but independent, effect was seen for smoking and black tea drinking indicating that a more complex model exists.

As questionnaires were performed with the participants, and AAO was self-reported, we cannot exclude the possibility of recall biases. However, after initial onset of motor symptoms in PD, patients likely seek medical advice relatively quickly. Hence, an older AAO would correlate with an older age at examination (AAE). To assess this type of recall bias, we compared the correlation between AAE and AAO in tobacco users and non-users (Supplementary Fig. 4). There were no differences in both groups (AAE and AAO were highly correlated, r > 0.8084, P < 0.0001). Although we cannot fully exclude all recall biases, we estimate that the recall bias for AAO is at least comparable in tobacco users and non-users and should not affect our analysis. Because the participants with LRRK2 p.Gly2019Ser were examined at the same center in Tunisia, selection bias from pooled participants examined at different centers was avoided, and consistency in diagnoses and reporting of clinical data was ensured. However, the participants are all from the same North African Arab-Berber origin, and lifestyle may be different in other populations. Further studies with LRRK2 mutation carriers from other populations are required. The collection of data was performed in-person on paper CRF forms followed by the transfer of data to an electronic database. Because not all participants had complete CRF data, we also cannot exclude potential biases. Socioeconomic status and other external factors may be a potential determinant of enrollment and collection of data. Moreover, the difficulties of participant enrollment can be largely explained by the Arab Spring. During the period of patient recruitment, political turmoil and the Tunisian revolution for on-site patient visits, along with tedious handwritten CRFs, has remained a hurdle.

The biological mechanisms that explain the protective effects of tobacco and caffeine still remain elusive, and we emphasize the importance of future molecular studies that aim to understand the underlying molecular mechanisms of neuroprotection.

Acknowledgments: Open access funding enabled and organized by Projekt DEAL.

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Neuropathological Findings in Ephedrine Encephalopathy

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ABSTRACT: Background: A number of cases of severe parkinsonism-dystonia have been recognized and reported following the illicit use of ephedrine prepared from pseudoephedrine and potassium permanganate. The pathology associated with ephedrine neurotoxicity has not been described yet in the scientific literature.

Objectives: To report the first neuropathological study of ephedrine toxicity.

Methods: The brain of a 33-year-old Ukrainian female ex-ephedrine addict with a long history of L-dopa-unresponsive parkinsonism with dystathria, dystonia, profound postural instability, cock-gait, and frequent falls, and on antiretroviral treatment, was examined using routine stains and immunohistochemistry.

Results: Neuropathological findings included diffuse pallidal astrogliosis without neuronal depletion. There was also widespread vascular pathology with small vessels occluded by foreign material, associated with giant cell response without any evidence of consequent focal infarction and a cerebellar abscess.

Conclusions: Clinical findings of L-dopa-unresponsive parkinsonism with dystonia, caused by illicit use of ephedrine, are fully consistent with neuropathological

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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Relevant conflicts of interest/financial disclosures: Nothing to report.

Full financial disclosures and author roles may be found in the online version of this article.

Accepted: 12 May 2020
Published online 16 June 2020 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28125