Neurosci. Bull. December, 2016, 32(6):572–576
DOI 10.1007/s12264-016-0070-5

www.neurosci.cn
www.springer.com/12264

REPORT

Olfactory Dysfunction in Parkinson's Disease Patients with the *LRRK2* G2385R Variant

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Received: 6 May 2016 / Accepted: 11 August 2016 / Published online: 3 October 2016

Abstract Olfactory dysfunction has been reported in Parkinson's disease (PD) patients carrying the *LRRK2* G2019S variant in Caucasians but rarely in those with the *LRRK2* G2385R variant. In this study, we performed genotyping for the *LRRK2* G2385R variant in PD patients recruited from the Movement Disorder Clinic of Xuanwu Hospital in Beijing and in healthy controls randomly selected from the Beijing Longitudinal Study on Aging cohort. The "five-odor olfactory detection array", an olfactory threshold test, was used to assess olfactory function. One hundred and eighty-six participants were enrolled, comprising 43 PD patients without (iPD) and 25 with (LRRK2-PD) the *LRRK2* G2385R variant, and 118 healthy controls. Our results showed that the threshold of olfactory identification was significantly worse in PD patients than in controls, but not significantly different between the iPD and LRRK2-PD groups. These findings suggested that although olfactory function in LRRK2-PD patients is impaired, it is similar to that in iPD patients.

Keywords Parkinson's disease · Olfactory dysfunction · *LRRK2* G2385R variant

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Introduction

Olfactory dysfunction is a common non-motor symptom among patients with Parkinson's disease (PD). About 75%–90% of PD patients present with hyposmia [1–3], suggesting that it is an early marker of developing PD [4]. However, a few studies have reported that olfactory function is less impaired in genetic PD with the leucine-rich repeat kinase 2 (*LRRK2*) G2019S variant than idiopathic PD in Caucasians [5–8], while little information is available on olfactory function in PD patients with the Asia-specific *LRRK2* G2385R variant. Previous studies have shown that the *LRRK2* G2385R variant is commonly associated with late-onset sporadic PD, which is clinically similar to idiopathic PD in Chinese patients [9, 10]. Here we carried out a preliminary study to investigate the effects of the *LRRK2* G2385R variant on olfaction in PD patients and healthy elderly controls.

Participants and Methods

Participants

PD patients were recruited from the Movement Disorders Clinic of Xuanwu Hospital of Capital Medical University in Beijing, China, and healthy controls were randomly selected from the Beijing Longitudinal Study on Aging cohort from 2013 to 2014 [11]. All participants were interviewed and examined by a movement disorder specialist. PD patients were diagnosed according to the UK Brain Bank Criteria [12]. Participants with incomplete clinical information, a positive neurological family history, cognitive impairment (Mini-Mental State Examination score < 24), rhinitis, a history of nasal disease, upper

respiratory infection (within two weeks), and nasal or head trauma were excluded from the study.

Before the beginning of the study, all participants signed the informed consent form, which was approved by the local Ethics Committee.

Olfactory Test

The "five-odor olfactory detection array", one of the Chinese olfactory tests, was applied [13–15]. The sensitivity of this test was 74.0% and the specificity was 91.7% as calculated in a previous study [16]. This test uses the following odors: acid (acetic acid), banana (amyl acetate), mint (menthol), flower (eugenol), and bad smell (scatole). The threshold of detection was determined with six concentrations of each odor at 1:10 dilution in liquid paraffin as the solvent. The odors were positioned from left to right in a box, arranged from low to high values, and each was labeled −2, −1, 0, 1, 2, and 3. Participants were settled in a quiet, ventilated room free of other odors. The test for each odor started from the lowest to the highest. The threshold of olfactory detection (TOD) was noted when the participant was able to smell the odor. The threshold of olfactory identification (TOI) was noted when the participant was able to give the correct name of the odor. The TOD and TOI scores were recorded on a standardized answer sheet. The score was recorded as 3.5 and labeled as anosmia if the participant could not identify any odor. The severity of olfactory dysfunction was represented by the mean value of the combination of TOD and TOI scores for all five odors, based on which the participants were classified as normal ($\leq$1), mild hyposmia (>1, $\leq$2), and severe hyposmia (>2) [13, 16].

Genotyping

LRRK2 genotyping was performed for all participants. The blood samples were analyzed as described previously [17]. The genotyping for the *LRRK2* G2385R variant (7153G>A, single nucleotide polymorphism [SNP] with accession no. rs34778348) was carried out with a single base primer extension assay using the ABI PRISM SNaP Shot Multiplex kit (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's recommendations. Analysis was carried out using Gene Mapper software (version 4.0 Applied Biosystems Inc., Foster City, CA). The primer sets used for assay of the G2385R variant were as follows: forward, TGCAATAGTCTAGCTTGTTT; reverse, GTGACACATGAAGTGCAA; SNP primer, GATAAGAAAACTGAAAAACTCTGT. Participants carrying the *LRRK2* G2385R variant were classified as *LRRK2* mutation carriers, while those without it, were defined as non-carriers.

Statistical Analysis

Data analysis was performed using SPSS 19.0 software (IBM Corp., New York, NY). Means and SDs were calculated for all continuous variables. The Mann–Whitney test was performed for pair-wise comparison of age, Hoehn and Yahr score [18], and the TOD and TOI scores, and the χ^2 test was used for pair-wise comparison of the frequencies of categorical variables. Logistic regression analysis was used to adjust for age, gender, and smoking in the comparison of severity of olfactory dysfunction among groups as well as for the variables TOD and TOI. A two-sided P value <0.05 was considered statistically significant.

Results

A total of 186 participants were enrolled, comprising 43 PD patients without *LRRK2* G2385R (iPD), 25 PD patients with the variant (LRRK2-PD), and 118 healthy participants without the variant. The demographic information is listed in Table 1. Healthy controls were slightly older and had fewer males than the PD groups. The TOD scores were similar among the iPD, LRRK2-PD, and control groups, but the TOI scores were significantly higher (worse) in both the iPD (2.58 ± 0.65 *vs* 2.36 ± 0.61, $P = 0.020$) and LRRK2-PD (2.70 ± 0.53 *vs* 2.36 ± 0.61, $P = 0.019$) groups than in controls (Table 1).

To investigate whether the *LRRK2* G2385R variant influences the severity of olfactory dysfunction, we compared the frequency of participants with severe olfactory dysfunction among the different groups (Table 2). We found that although the proportion of participants with severe olfactory dysfunction was only slightly but not significantly different between the iPD and LRRK2-PD groups (96.0% *vs* 86.0%), the proportion in both iPD and LRRK2-PD patients was significantly higher than that in controls (Table 2).

Furthermore, the TOD differed between the iPD and control groups (1.90 ± 1.33 *vs* 1.21 ± 1.22, $P = 0.004$) and the TOI differed between the LRRK2-PD and control groups (2.54 ± 1.44 *vs* 2.11 ± 1.29, $P = 0.045$). The TOI scores for Banana and Mint were significantly different between the PD and control groups (Banana: LRRK2-PD *vs* control, 3.22 ± 0.52 *vs* 2.40 ± 1.04, $P = 0.0001$; Mint: iPD *vs* control, 2.89 ± 0.81 *vs* 2.40 ± 0.98, $P = 0.005$; LRRK2-PD *vs* control, 3.22 ± 0.50 *vs* 2.40 ± 0.98, $P = 0.0001$), but interestingly, the TOI score for Banana was much higher (worse) in LRRK2-PD than in iPD patients (3.22 ± 0.52 *vs* 2.57 ± 0.98, $P = 0.015$). Also, the TOI scores for Flower differed between the iPD and control groups (2.49 ± 0.79 *vs* 2.74 ± 1.03, $P = 0.032$).

Table 1 Demographics of PD patients and controls

	LRRK2-PD ($n = 25$)	iPD ($n = 43$)	Control ($n = 118$)	LRRK2-PD *vs* iPD *P*1 (adjusted P1)	iPD *vs* control *P*2 (adjusted P2)	LRRK2-PD *vs* control *P*3 (adjusted P3)
Age (mean ± SD)	68.12 ± 10.05	65.89 ± 10.93	71.12 ± 8.51	0.356	0.001	0.121
Gender, male (%)	16 (64.0)	28 (65.1)	49 (41.5)	0.926	0.008	0.040
H&Y	2.2 ± 0.80	2.4 ± 0.74	/	0.232	/	/
Smoking [*n*, (%)]	8/22 (36.4)	12/36 (33.3)	43/109 (39.4)	0.814	0.512	0.787
TOD (mean ± SD)	1.42 ± 1.20	1.49 ± 1.00	1.24 ± 0.93	0.868 (0.608)	0.093 (0.296)	0.296 (0.687)
TOI (mean ± SD)	2.70 ± 0.53	2.58 ± 0.65	2.36 ± 0.61	0.289 (0.867)	0.071 (0.020)	0.017 (0.019)

P values in pair-wise comparisons were made using the Mann–Whitney test and adjusted *P* values were adjusted by multivariate linear regression analysis for age, gender, and smoking

H&Y Hoehn and Yahr score

Table 2 Distribution of olfactory dysfunction in PD patients and controls

	LRRK2-PD ($n = 25$)	iPD ($n = 43$)	Control ($n = 118$)	LRRK2-PD *vs* iPD *P*1, OR (95% CI)	iPD *vs* control *P*2, OR (95% CI)	LRRK2-PD *vs* control *P*3, OR (95% CI)
Normal/mild olfactory dysfunction [*n*, (%)]	1 (4.0%)	6 (14.0%)	36 (30.5%)	Ref	Ref	Ref
Severe olfactory dysfunction [*n*, (%)]	24 (96.0%)	37 (86.0%)	82 (69.5%)	0.221 3.892(0.441–34.375)	0.021 3.921(1.233–12.466)	0.018 12.725(1.556–104.029)

*P*1 values were not adjusted because of the small sample size ($n = 7$) in the normal/mild olfactory dysfunction group; *P*2 and *P*3 values were adjusted by logistic regression analysis for age, gender, and smoking

ref reference

Discussion

Here, we confirmed that olfactory dysfunction in Chinese participants, especially the threshold of odor identification, was impaired in both iPD and LRRK2-PD patients compared to controls. This finding is in agreement with the majority of reports on Caucasian participants [1, 19]. Previous studies have shown that olfactory function presents better UPSIT (University of Pennsylvania Smell Identification Test) scores in *LRRK2* G2019S PD patients than in iPD patients, indicating that this variant might preserve olfactory function in PD patients [8] and patients with this variant might have a different subtype of PD [20, 21]. However, we did not obtain the same result in PD patients with the *LRRK2* G2385R variant. Both the TOI and TOD scores of LRRK2-PD patients were similar to those in iPD patients. Even when the olfactory dysfunction was classified as mild or severe, the proportion of severe olfactory dysfunction did not differ significantly between the LRRK2-PD and iPD groups. Whether the discrepancy in olfactory dysfunction between *LRRK2* G2019S and G2385R variants results from mutation-specific effects or aging warrants further investigation.

Olfactory dysfunction might be selective for specific odors, as has been suggested in studies of the Caucasian population [22, 23]. One previous study from Germany reported that licorice, banana, aniseed, pineapple, apple, and turpentine are the best odors for discriminating PD from controls [24], while an Australian study suggested that the five odors pineapple, banana, gasoline, smoke, and cinnamon have the best discriminatory value for PD [25]. One American study found that the three odors banana, licorice, and dill pickle have high accuracy for the diagnosis of PD [22]. Our study found that the odors of Banana and Mint had better specificity in identifying olfactory dysfunction in Chinese PD patients. Interestingly, the odor of Banana (amyl acetate) was significantly worse in terms of TOI score in LRRK2-PD than iPD patients. Whether the inability to identify amyl acetate might be associated with *LRRK2* mutations needs further study.

It is known that the *LRRK2* G2385R variant is associated with susceptibility to PD in East-Asians [26] and its clinical features are similar to those of iPD patients [9]. However, less information on the pathology of PD associated with the *LRRK2* G2385R variant has been reported. According to previous studies, α-synuclein deposits are present in the olfactory bulb and anterior olfactory nucleus at Braak stage I [27, 28], providing evidence that PD might initiate from the olfactory system at the pathological level [29]. Studies have shown that PD patients with the *LRRK2*

G2019S mutation have Lewy body pathology in most cases [30], including α-synuclein aggregation in the rhinencephalon [31], suggesting that *LRRK2* mutations affect the olfactory system. Future studies on the pathology of PD associated with the *LRRK2* G2385R variant may clarify whether olfactory dysfunction is associated with α-synucle in pathology in the olfactory system (olfactory epithelium, olfactory bulb, and piriform cortex).

The present study has some limitations, such as the small sample size and genotyping only for the *LRRK2* G2385R variant. The age and gender of participants in the PD (LRRK2-PD and iPD) and control groups were not well-matched though they were adjusted for. Last but not least, a longitudinal follow-up or a large-scale clinical study is needed to replicate our findings.

In summary, olfactory impairment is common in PD patients, including those with the *LRRK2* G2385R variant. PD patients with this variant have olfactory dysfunction similar to iPD patients.

Acknowledgements This work was supported by grants from the Scientific Project of Ministry of Science and Technology of China (2012AA02A514), the National Basic Research Development Program of China (2011CB504101), and the Beijing High Standard Health Human Resource Cultural Program in Health System, China (2009e1e12).

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