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Olfactory Dysfunction in Parkinsonism Caused by *PINK1* Mutations

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Abstract: Hyposmia is a common nonmotor feature of Parkinson's disease (PD) and has been variably detected in monogenic Parkinsonisms. To assess olfactory dysfunction in *PINK1*-related Parkinsonism, we evaluated olfactory detection threshold, odor discrimination, and odor identification in five groups of subjects: sporadic PD (n = 19), *PINK1* homozygous (n = 7), and heterozygous (n = 6) parkinsonian patients, asymptomatic *PINK1* heterozygous carriers (n = 12), and Italian healthy subjects (n = 67). All affected subjects and all healthy heterozygotes but one resulted hyposmic, with most patients in the range of functional anosmia or severe hyposmia. Detection threshold was more preserved and discrimination more impaired in patients with *PINK1* mutations than in PD cases. Alterations of detection and discrimination were observed also in *PINK1* asymptomatic heterozygotes. On the contrary, odor identification appeared to be mostly related to the disease status, as it was impaired in nearly all patients (including PD and *PINK1* cases) and preserved in healthy heterozygotes. Our data indicate that olfactory dysfunction is common in *PINK1* Parkinsonism and consists typically in defective odor identification and discrimination. A milder olfactory deficit, mostly involving discrimination, can be found in asymptomatic heterozygotes, possibly indicating an underlying preclinical neurodegenerative process. © 2009 Movement Disorder Society

Key words: Parkinson's disease; olfaction; *PINK1*; Sniffin' sticks; hyposmia

Olfactory dysfunction is one of the commonest nonmotor features of Parkinson's disease (PD). A recent comprehensive assessment of different olfactory abilities, namely odor threshold, discrimination, and identification, in 400 patients with PD indicated that upto 97% of them could be defined hyposmic when compared with young normosmic subjects, and 75% still remained hyposmic after adjustment to age-related normatives.[1] The olfactory deficit is bilateral, does not respond to dopaminergic therapy, and usually appears early in the disease course or even before the onset of motor symptoms.[2–8] Indeed, a population-based prospective study demonstrated that olfactory deficit bears an increased risk (upto five-fold) to develop PD and can precede the diagnosis by at least 4 years.[9]

The pathophysiological basis of the olfactory dysfunction in PD is still debated, although the presence of Lewy bodies and Lewy neurites has been described

Additional Supporting Information may be found in the online version of this article.

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TABLE 1. *Demographic and clinical features of the five groups of subjects*

	PD (n = 19)	A-Het (n = 6)	Hom (n = 7)	H-Het (n = 12)	CTR (n = 67)
Male	11 (58%)	3 (50%)	5 (71%)	10 (83%)	34 (51%)
Age (yr)	68.7 ± 7.0 (55; 79)	65.5 ± 7.9 (57; 76)	59.9 ± 13.1 (47; 81)	43.7 ± 19.7 (26; 79)	43.2 ± 17.8 (16; 87)
Age at onset (yr)	62.4 ± 8.2 (49; 77)	54.3 ± 7.3 (46; 66)	38.6 ± 6.4 (30; 48)	na	na
Disease duration (yr)	6.4 ± 3.8 (2; 16)	11.2 ± 3.5 (6; 16)	21.3 ± 9.2 (9; 36)	na	na
MMSE	28.2 ± 1.8 (24; 30)	28.8 ± 1.6 (26; 30)	28.9 ± 1.7 (26; 30)	28.9 ± 1 (27; 30)	na
UPDRSIII on	19.9 ± 8.1 (6; 34)	14.2 ± 7.1 (5; 26)	17.7 ± 9.9 (6; 31)	na	na
UPDRSIII off	27.5 ± 9.7 (1; 46)	25.7 ± 9.5 (15; 33)	40.3 ± 8.7 (25; 49)	na	na
Hoehn-Yahr off	2.5 ± 0.8 (1; 4)	2.8 ± 0.4 (2; 3)	2.7 ± 1.1 (1; 4)	na	na
Smokers (%)	1 (5%)	1 (17%)	3 (43%)	4 (33%)	17 (25%)
Subjective hyposmia	13 (68%)	6 (100%)	5 (71%)	1 (8%)	0

Quantitative values are presented as mean ± standard deviation and range (min; max).
MMSE, mini mental state examination; UPDRSIII, unified Parkinson's disease rating score motor section; na, not applicable.

in several areas implicated in the olfactory system, such as the olfactory bulb, the anterior olfactory nucleus, and the amygdala.[10,11]

In most studies, the assessment of olfactory performance and the subsequent diagnosis of hyposmia have been based on the University of Pennsylvania Smell Identification Test (UPSIT) or other similar psychophysical methods, that specifically assess odor identification. Overall, this ability was reported to be impaired in 60–96% patients with PD.[1,3,12–17] Detection threshold and odor discrimination have been less frequently evaluated, and in several studies, they resulted abnormal in smaller proportions of patients (about 45–83% and 34–87%, respectively).[1,14,17–19] Of these abilities, only odor discrimination has been related to either disease severity[14] or duration[17] with discordant results, while identification has been repeatedly shown not to correlate with either.[3,14–17]

Olfactory dysfunction has been considered as a possible tool to differentiate PD from other parkinsonian syndromes (reviewed in[20,21]), but only few studies have explored olfactory abilities (mainly odor identification) in monogenic Parkinsonisms. At difference from PD, identification appeared to be consistently preserved in autosomal recessive Parkinsonism (ARP) caused by mutations in *PARK2/Parkin* or *DJ-1* genes,[22,23] while it was variably affected in autosomal dominant forms due to mutations in *LRRK2* or *SNCA* genes.[24–28] Odor discrimination and detection threshold were tested only in six ARP patients (five mutated in *Parkin* and one in *DJ-1*) and in seven *SNCA* mutated cases, with variable results.[23,25] Data on specific olfactory abilities in monogenic Parkinsonisms are detailed in Supporting Information Table 1.

PINK1 mutations represent the second most frequent cause of ARP after *Parkin*.[29–32] The typical *PINK1*-associated phenotype is characterized by early age at onset, slow disease progression, and excellent response to levodopa; yet in rare cases, the clinical presentation can be indistinguishable from PD.[33] Heterozygous rare variants in the *PINK1* gene, as well as in other ARP genes, have been frequently detected both in parkinsonian patients and in healthy controls. Although their pathogenetic role is still debated, these variants have been suggested to act as minor risk factors to develop PD.[31,34]

To our knowledge, a detailed evaluation of the distinct olfactory abilities in *PINK1*-related Parkinsonism has not been performed to date, and only a single *PINK1* homozygous patient was tested for odor identification that resulted to be impaired.[35] Here, we adopted the Sniffin' Sticks test to individually explore detection threshold, odor identification, and discrimination in patients with homozygous and heterozygous *PINK1* mutations, and results were compared with those obtained in two groups of PD cases and healthy controls. Since *PINK1* heterozygous mutations have been previously associated with subclinical signs of dopaminergic dysfunction in asymptomatic carriers,[36] a group of these subjects also underwent olfactory testing.

SUBJECTS AND METHODS

Subjects

Five groups of subjects were included in the study: (1) Hom (n = 7): six affected subjects homozygous for the W437X mutation[37] and a sporadic case homo-

zygous for the A168P mutation[38]; (2) H-Het (n = 12): asymptomatic relatives of Hom patients, heterozygous for either W437X (n = 10) or A168P (n = 2); (3) A-Het (n = 6): heterozygous carriers of a single *PINK1* rare variant (L67F, I111S, P322L, g.15445_15467del23, E476K, D525N)[34]; (4) PD (n = 19): sporadic late onset PD; (5) CTR (n = 67): Italian healthy controls. Both PD and CTR subjects tested negative for the common *LRRK2* G2019S mutation and for mutations in the *PINK1* gene.

All subjects were interviewed and examined by a neurologist expert in movement disorders, who diagnosed PD in accordance with the UK Brain Bank Criteria[39] and ascertained the absence of mild parkinsonian signs or other movement disorders in asymptomatic heterozygotes. Basic demographic and clinical features in the five groups of subjects are summarized in Table 1. Each subject gave written informed consent to participate in the study, that was approved by the Ethic Committee of the Catholic University of Rome.

Olfactory Test

To avoid biases related to nasal obstruction or dysfunction, nasal function was assessed in all subjects before the olfactory test. The volume and transversal areas of nasal cavity were first assessed by acoustic rhinometry. Subjects with values indicative of nasal congestion or obstruction were treated with chlorohydrate tramazoline and a new rhinometry was performed after 10 minutes. A test assessing mucociliary transport time was also performed to evaluate eutrophism of the nasal mucosa.[40]

The Sniffin' Sticks test (Burghart, Wedel, Germany) was administered to all participating subjects, with the investigator blinded to the individual *PINK1* genotype. Odors were presented in batteries of felt-tip pens filled with liquid odorants. The test involved three separate tasks to evaluate odor perception threshold (OT), odor discrimination (OD: ability to discriminate among different odors), and odor identification (OI: ability to identify specific odors). Each task generated a raw score ranging from 0 to 16, with 16 indicating the best performance. A composite raw TDI score was calculated from the sum of the three task-specific scores (range: 0–48).[41]

Data Analysis

According to the raw TDI score, each subject was first assigned to one of the following categories: normosmia ($TDI > 30$); mild hyposmia ($25 < TDI \leq 30$); moderate hyposmia ($20 < TDI \leq 25$); severe hyposmia ($15 < TDI \leq 20$); functional anosmia ($TDI \leq 15$). A general linear model (GLM) multivariate analysis was then performed using the SPSS v16.0 software (SPSS, Chicago, IL), to test for differences of the four olfactory scores (set as variables) among groups of subjects (set as factor), assuming age, gender, and smoke habit as covariates for regression analysis. This procedure also provided univariate analysis of variance for each dependent variable. Estimated marginal means with 95% confidence intervals were calculated to give estimates of predicted mean values for each combination of variables and factors in the model. To investigate the hypothesis that *PINK1* mutation carriers might have different olfactory performances compared with PD and CTR groups, post hoc pairwise comparisons were also performed, using the Bonferroni correction for multiple tests.

Finally, to describe the olfactory performance in each individual, each score was transformed according to the following formula, adapted from *Z*-score normalization:

$$nS = (S - Mctr)/SDctr$$

where nS is the individual normalized score (nTDI, nOT, nOD, or nOI), *S* the individual raw score (TDI, OT, OD, or OI), and Mctr and SDctr are the mean value and the standard deviation from the age-matched subgroup of controls. For this purpose, controls were divided in three age categories: 16–35, 36–55, and over 55 years.[42] Applying this formula, each individual value corresponding to the mean and 1 SD of the matched control subgroup is always set at 0 and 1, respectively. The normalized value of -2, corresponding to -2 SDs from the age-matched control mean, was used to define the normal limit of each olfactory score.

RESULTS

A subjective decline of olfactory function was reported by the majority of patients, compared with one healthy heterozygote (Table 1). No difficulties were experienced while performing the test, and nasal function was normal in all subjects.

TDI scores ≤ 30 were found in all parkinsonian subjects and all healthy heterozygotes but one (Fig. 1). All parkinsonian patients were hyposmic also in relation to age, with most cases falling in the range of functional anosmia or severe hyposmia. Eleven of 12 healthy heterozygotes resulted mildly to moderately hyposmic, but only eight were pathologic in relation to their age.

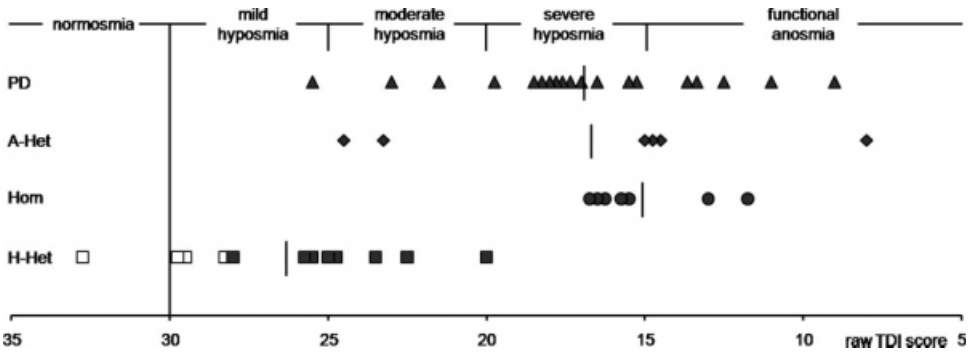

FIG. 1. Raw TDI scores. White and gray symbols represent individual raw TDI values that are normal or pathological for age (> or ≤ −2SD of normal age-matched controls), respectively. Vertical bars show mean values for each group. PD, Parkinson's disease; Hom, *PINK1* homozygous patients; A-Het and H-Het, affected and healthy subjects with *PINK1* heterozygous mutations.

Mean raw olfactory scores for each group of subjects are presented in Table 2.

Multivariate statistical analysis using TDI, OT, OD, OI as dependent variables, the groups as factor, and age, gender, and smoke habit as covariates, revealed significant differences between groups ($P < 0.0001$). Age contributed less ($P < 0.005$), while gender and smoke did not significantly contribute to the model. Univariate analysis indicated that the effect of the factor "groups" was significant on each olfactory measure ($P < 0.0001$). Profiles of estimated means of TDI, OD, OT, and OI in the five groups (adjusted for age, gender, and smoke habit) are shown in Figure 2.

Compared with controls, all parkinsonian groups showed a significant decline of all estimated mean scores. Healthy heterozygotes also had significantly reduced means for TDI, OT, and OD scores, but were comparable to controls regarding OI. Compared with PD, the A-Het showed comparable means for each scores except OD that was overall impaired in both groups, but more severely in A-Het. Conversely, homozygotes significantly differed from the PD group in each ability, with an olfactory deficit that was more severe for OD and OI and milder for OT. Of note, the three *PINK1* mutated groups (including affected and healthy subjects) had comparable estimated means for both OT and OD, while they could be clearly discriminated based on their OI means (Hom < A-Het < H-Het).

The normalized individual scores (nTDI, nOT, nOD, and nOI) are presented in Figure 3 and allow a direct comparison of each subject with age-matched normative data, any value ≤−2 indicating a pathological result falling at or below −2 SD of matched controls

TABLE 2. *Raw and normalized olfactory scores from the five groups of subjects*

	PD (n = 19)	A-Het (n = 6)	Hom (n = 7)	H-Het (n = 12)	CTR (n = 67)
TDI	16.9 ± 4.0 (9.0; 25.5)	16.7 ± 6.2 (8.0; 24.5)	15.0 ± 1.9 (11.8; 16.5)	26.3 ± 3.5 (20.0; 32.8)	35.5 ± 4.6 (27.2; 46.5)
OT	2.6 ± 1.6 (1.0; 6.5)	5.2 ± 4.4 (1.0; 13.5)	7.5 ± 3.3 (1.0; 11.5)	7.0 ± 3.1 (3.5; 12.0)	10.8 ± 2.1 (6.5; 15.5)
OD	7.7 ± 2.1 (4; 12)	4.3 ± 1.2 (3; 6)	4.3 ± 2.4 (2; 7)	6.3 ± 2.9 (1; 10)	12.0 ± 2.4 (6; 16)
OI	6.6 ± 1.6 (4; 10)	7.3 ± 4.1 (3; 15)	3.4 ± 1.3 (2; 6)	13.0 ± 1.4 (10; 15)	12.6 ± 2.1 (9; 16)
nTDI	−4.6 ± 1.2 (−6.9; −2.1)	−4.7 ± 1.8 (−7.2; −2.4)	−5.3 ± 0.6 (−6.5; −4.8)	−2.3 ± 0.9 (−4.2; −1.0)	0 ± 1 (−1.6; +1.8)
nOT	−5.6 ± 1.2 (−6.9; −2.9)	−3.9 ± 3.2 (−6.9; +2.1)	−2.1 ± 2.4 (−6.9; +0.4)	−1.9 ± 1.6 (−4.7; +0.4)	0 ± 1 (−1.8; +2.7)
nOD	−1.3 ± 0.9 (−3.0; +0.7)	−2.9 ± 0.6 (−3.5; −2.1)	−3.0 ± 1.2 (−4.3; −1.6)	−2.9 ± 1.6 (−6.3; −0.9)	0 ± 1 (−2.2; +1.6)
nOI	−2.5 ± 0.8 (−3.7; −0.9)	−2.1 ± 2.0 (−4.2; +1.6)	−4.2 ± 0.8 (−5.2; −2.8)	0.1 ± 0.9 (−0.9; +1.6)	0 ± 1 (−1.9; +2.0)

Data are presented as mean ± standard deviation and range (min; max). OT, OD, OI, TDI, raw scores for olfactory threshold, discrimination, identification, and the combined TDI value; nOT, nOD, nOI, nTDI, age-normalized scores.

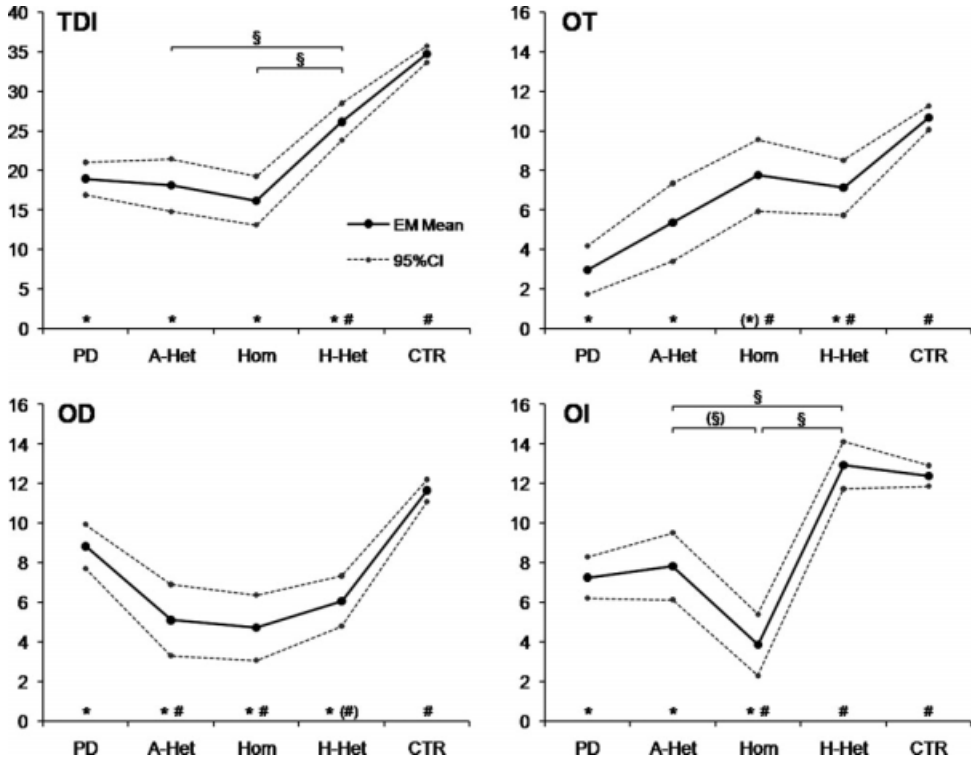

FIG. 2. Estimated mean scores. Estimated marginal (EM) means and 95% confidence interval (CI) lower and upper bounds calculated using the GLM multivariate analysis in the five groups of tested subjects. Pairwise post hoc comparisons against CTR or PD groups that reached significance after correction for multiple tests are indicated with symbols * and $^{\#}$($P < 0.005$ against CTR and PD, respectively), or symbols $^{(*)}$ and $^{(\#)}$ ($P < 0.05$). Additional significant pairwise comparisons are indicated with symbols §($P < 0.005$) or $^{(\S)}$($P < 0.05$).

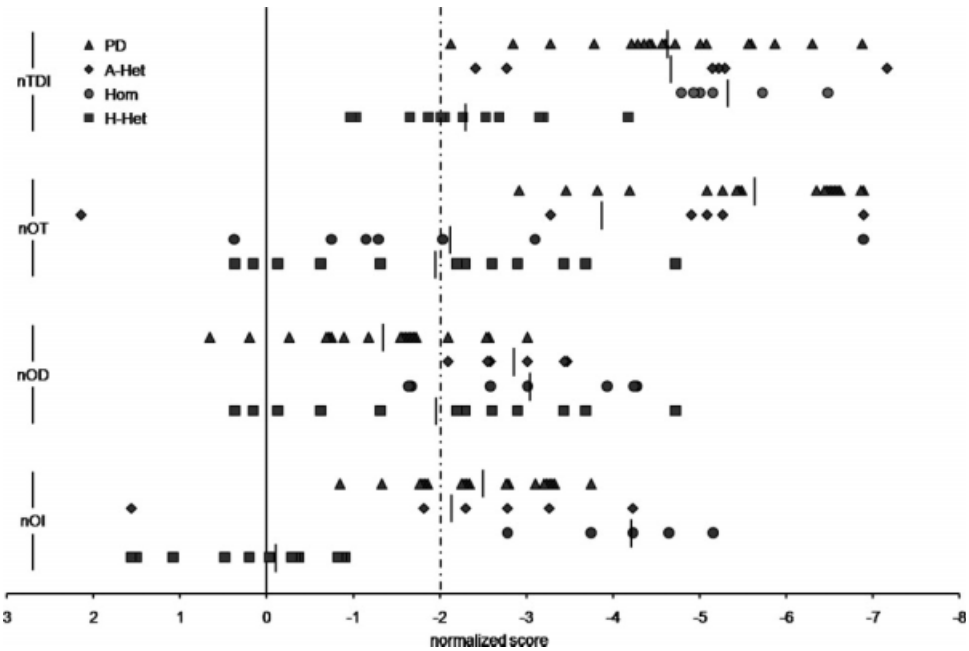

FIG. 3. Age-normalized scores. Normalized control means and SD are set to reference values of 0 and 1, respectively. Each individual normalized score ≤ -2 indicates a pathological olfactory score ($\leq$–2 SDs of age-matched controls). Vertical bars represent mean values. nOT, nOD, nOI, nTDI, age-normalized scores for the olfactory threshold, discrimination, identification, and the combined TDI value.

(Fig. 3). Table 2 summarizes mean normalized scores for all tested groups.

No obvious correlation emerged between each olfactory ability and either disease duration or severity (UPDRSIII and Hoehn-Yahr score) in the three patients' groups.

DISCUSSION

We report the first assessment of multiple olfactory abilities in the Parkinsonism caused by mutations in the *PINK1* gene. Most published studies of olfactory dysfunction in *SNCA, LRRK2*, or *Parkin* related Parkinsonism, as well as in a single *PINK1* patient, were limited to the assessment of odor identification, and only few cases have been additionally tested for either detection threshold or odor discrimination (Supporting Information Table 1).[23,25] In this study, the use of the Sniffin' Sticks test allowed to perform a detailed assessment of these three abilities through individual scores that could be summed to obtain an overall evaluation of the olfactory dysfunction (TDI).

All *PINK1* homozygotes had TDI scores clearly below the cut-off value of 30, which is generally used to define the limit of normality, indicating a condition of severe hyposmia or functional anosmia.[42] Moreover, individual values were all below −2SD of Italian age-matched controls, and the mean was significantly lower than in controls also after correction for age, gender, and smoke habit. These findings indicate that olfactory loss is a consistent feature of *PINK1*-ARP. TDI scores were similarly reduced also in parkinsonian *PINK1* heterozygotes and patients with PD, indicating that this global score can be a sensitive parameter to detect a general olfactory dysfunction but is not effective in discriminating among the three tested subgroups. Of note, most clinically asymptomatic *PINK1* heterozygotes also presented an overall olfactory performance that was either toward the lowest normal limit or pathological, although the mean TDI reduction was milder than in the parkinsonian cohorts.

The most striking differences between groups emerged when specific tasks were analyzed independently. The advantage of exploring multiple olfactory abilities had been already demonstrated in a recent study on 52 patients with PD and 50 controls tested by Sniffin' Sticks, in which this extended strategy clearly improved the diagnostic accuracy of the test. In particular, the combination of odor identification and detection threshold was shown to bear the highest sensitivity and specificity in distinguishing patients with PD from controls.[43] In our study, mean identification was abnormal in all groups of patients, although considerably lower in *PINK1* homozygotes than in the two other groups, while it was entirely normal in healthy heterozygotes. This finding is in line with data from the only *PINK1* homozygous patient reported so far, who also presented abnormal identification,[35] and with published literature that clearly indicates this specific ability as the most frequently altered in PD. At difference from *PINK1*-Parkinsonism and PD, mutations in the *Parkin* gene appear to be consistently associated with preserved identification, evaluated either with UPSIT or Sniffin' Sticks.[22,23] Therefore, in a parkinsonian patient, olfactory tests selectively exploring odor identification might be supportive in discriminating PD from *Parkin*- but not from *PINK1*-related Parkinsonism.

Relevant data also came from the analysis of the other two olfactory abilities. In fact, mean detection threshold was more severely affected in PD than in all *PINK1* groups. Conversely, mean discrimination was markedly impaired in all *PINK1*-mutated groups, including patients and healthy heterozygotes. On average, patients with PD also showed a discrimination deficit, that, however, was significantly milder than in all *PINK1* cohorts. This impairment of odor discrimination consistently seen in affected *PINK1* mutation carriers, but common also in healthy carriers is remarkable. In a PET neuroimaging study that explored the activation of distinct cerebral areas during specific olfaction-related tasks, odor discrimination was found to selectively activate the right caudate nucleus and right subiculum-hyppocampus, whereas other brain areas such as right amygdala, right piriform and orbitofrontal cortices, right thalamus, right cerebellum, and left insula could be recruited also by other olfactory tasks.[44] Interestingly, PINK1 is known to be considerably expressed in all these regions, including those specific for odor discrimination,[45] suggesting a possible important role of this protein in regulating neuronal function in these brain areas. A patent discrimination deficit was observed also in *PINK1* healthy heterozygotes, suggesting that this defect appears early and tends to remain stable over time. Of note, a previous PET investigation in three *PINK1* healthy heterozygotes with olfactory deficit had revealed a mild but significant nigrostriatal dopaminergic dysfunction, consistent with an underlying subclinical neurodegenerative process.[36]

It has been suggested that olfactory dysfunction might be related to the presence of Lewy bodies and to alpha-synucleinopathy. The preserved identification ability reported in *Parkin* homozygotes, who generally

lack Lewy body pathology, would support this hypothesis; on the other hand, this is weakened by the report of some patients with *SNCA*-related Parkinsonism (a typical alpha-synucleinopathy with Lewy bodies) who present normal odor detection and identification.[25] Classical Lewy bodies have been found in the brains of four *PINK1* heterozygous patients,[46] but to date no neuropathological data are available for homozygotes or compound heterozygotes. In these cases, the assessment of Lewy bodies would be of great relevance in light of the present olfactory findings.

In conclusion, our study demonstrates that the olfactory deficit is a consistent feature of *PINK1*-related Parkinsonism and suggests that the olfactory performance in this parkinsonian syndrome may differ both from *Parkin* disease, that lacks the identification deficit, and from sporadic PD, that overall shows more severe impairment of threshold and less severe discrimination deficit than in *PINK1* disease.

These results highlight the importance of testing distinct abilities to disclose specific patterns of olfaction dysfunction. Along with other clinical and instrumental findings, these profiles could help differentiate among different parkinsonian syndromes and establish relevant correlations with neuropathological features. Further studies on larger cohorts of patients are needed to assess the specificity and sensitivity of these tests, to make their application useful also in clinical practice.

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Author Roles: Alessandro Ferraris, Giulio Cesare Passali, Enza Maria Valente, and Anna Rita Bentivoglio were involved in the conception and execution of the project, data and statistical analysis, and manuscript writing. Tamara Ialongo, Marianna Laruffa, Arianna Guidubaldi, Maria Teresa Pellecchia, and Livia Brusa were involved in the organization and execution of the project. Gaetano Paludetti, Alberto Albanese, Paolo Barone, and Bruno Dallapiccola were involved in the critical revision of data and revision of the manuscript for intellectual content. EMV and ARB had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Haehner A, Boesveldt S, Berendse HW, et al. Prevalence of smell loss in Parkinson's disease—a multicenter study. Parkinsonism Relat Disord 2009;15:490–494.
2. Quinn NP, Rossor MN, Marsden CD. Olfactory threshold in Parkinson's disease. J Neurol Neurosurg Psychiatry 1987;50:88–89.
3. Doty RL, Deems DA, Stellar S. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. Neurology 1988;38:1237–1244.
4. Doty RL, Stern MB, Pfeiffer C, Gollomp SM, Hurtig HI. Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1992;55:138–142.
5. Berendse HW, Booij J, Francot CM, et al. Subclinical dopaminergic dysfunction in asymptomatic Parkinson's disease patients' relatives with a decreased sense of smell. Ann Neurol 2001;50:34–41.
6. Sommer U, Hummel T, Cormann K, et al. Detection of presymptomatic Parkinson's disease: combining smell tests, transcranial sonography, and SPECT. Mov Disord 2004;19:1196–1202.
7. Ponsen MM, Stoffers D, Booij J, Van Eck-Smit BL, Wolters EC, Berendse HW. Idiopathic hyposmia as a preclinical sign of Parkinson's disease. Ann Neurol 2004;56:173–181.
8. Haehner A, Hummel T, Hummel C, Sommer U, Junghanns S, Reichmann H. Olfactory loss may be a first sign of idiopathic Parkinson's disease. Mov Disord 2007;22:839–842.
9. Ross GW, Petrovitch H, Abbott RD, et al. Association of olfactory dysfunction with risk for future Parkinson's disease. Ann Neurol 2008;63:167–173.
10. Pearce RK, Hawkes CH, Daniel SE. The anterior olfactory nucleus in Parkinson's disease. Mov Disord 1995;10:283–287.
11. Harding AJ, Stimson E, Henderson JM, Halliday GM. Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. Brain 2002;125:2431–2445.
12. Stern MB, Doty RL, Dotti M, et al. Olfactory function in Parkinson's disease subtypes. Neurology 1994;44:266–268.
13. Hawkes CH, Shephard BC. Olfactory evoked responses and identification tests in neurological disease. Ann NY Acad Sci 1998;855:608–615.
14. Tissingh G, Berendse HW, Bergmans P, et al. Loss of olfaction in de novo and treated Parkinson's disease: possible implications for early diagnosis. Mov Disord 2001;16:41–46.
15. Double KL, Rowe DB, Hayes M, et al. Identifying the pattern of olfactory deficits in Parkinson disease using the brief smell identification test. Arch Neurol 2003;60:545–549.
16. Kim JY, Lee WY, Chung EJ, Dhong HJ. Analysis of olfactory function and the depth of olfactory sulcus in patients with Parkinson's disease. Mov Disord 2007;22:1563–1566.
17. Boesveldt S, Verbaan D, Knol DL, et al. A comparative study of odor identification and odor discrimination deficits in Parkinson's disease. Mov Disord 2008;23:1984–1990.
18. Ansari KA, Johnson A. Olfactory function in patients with Parkinson's disease. J Chronic Dis 1975;28:493–497.
19. Ward CD, Hess WA, Calne DB. Olfactory impairment in Parkinson's disease. Neurology 1983;33:943–946.
20. Hawkes C. Olfaction in neurodegenerative disorder. Mov Disord 2003;18:364–372.
21. Katzenschlager R, Lees AJ. Olfaction and Parkinson's syndromes: its role in differential diagnosis. Curr Opin Neurol 2004;17:417–423.

22. Khan NL, Katzenschlager R, Watt H, et al. Olfaction differentiates parkin disease from early-onset parkinsonism and Parkinson disease. Neurology 2004;62:1224–1226.
23. Verbaan D, Boesveldt S, Van Rooden SM, et al. Is olfactory impairment in Parkinson disease related to phenotypic or genotypic characteristics? Neurology 2008;71:1877–1882.
24. Markopoulou K, Larsen KW, Wszolek EK, et al. Olfactory dysfunction in familial parkinsonism. Neurology 1997;49:1262–1267.
25. Bostantjopoulou S, Katsarou Z, Papadimitriou A, Veletza V, Hatzigeorgiou G, Lees A. Clinical features of parkinsonian patients with the alpha-synuclein (G209A) mutation. Mov Disord 2001; 16:1007–1013.
26. Ferreira JJ, Guedes LC, Rosa MM, et al. High prevalence of LRRK2 mutations in familial and sporadic Parkinson's disease in Portugal. Mov Disord 2007;22:1194–1201.
27. Healy DG, Falchi M, O'Sullivan SS, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol 2008;7:583–590.
28. Silveira-Moriyama L, Guedes LC, Kingsbury A, et al. Hyposmia in G2019S LRRK2-related parkinsonism: clinical and pathologic data. Neurology 2008;71:1021–1026.
29. Valente EM, Salvi S, Ialongo T, et al. PINK1 mutations are associated with sporadic early-onset parkinsonism. Ann Neurol 2004; 56:336–341.
30. Ibanez P, Lesage S, Lohmann E, et al. Mutational analysis of the PINK1 gene in early-onset parkinsonism in Europe and North Africa. Brain 2006;129:686–694.
31. Kumazawa R, Tomiyama H, Li Y, et al. Mutation analysis of the PINK1 gene in 391 patients with Parkinson disease. Arch Neurol 2008;65:802–808.
32. Ishihara-Paul L, Hulihan MM, Kachergus J, et al. PINK1 mutations and parkinsonism. Neurology 2008;71:896–902.
33. Gelmetti V, Ferraris A, Brusa L, et al. Late onset sporadic Parkinson's disease caused by PINK1 mutations: clinical and functional study. Mov Disord 2008;23:881–885.
34. Marongiu R, Ferraris A, Ialongo T, et al. PINK1 heterozygous rare variants: prevalence, significance and phenotypic spectrum. Hum Mutat 2008;29:565–577.
35. Doostzadeh J, Tetrud JW, Len-Auerbach M, Langston JW, Schule B. Novel features in a patient homozygous for the L347P mutation in the PINK1 gene. Parkinsonism Relat Disord 2007;13:359–361.
36. Khan NL, Valente EM, Bentivoglio AR, et al. Clinical and subclinical dopaminergic dysfunction in PARK6-linked parkinsonism: an 18F-dopa PET study. Ann Neurol 2002;52:849–853.
37. Valente EM, Abou-Sleiman PM, Caputo V, et al. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 2004;304:1158–1160.
38. Albanese A, Valente EM, Romito LM, Bellacchio E, Elia AE, Dallapiccola B. The PINK1 phenotype can be indistinguishable from idiopathic Parkinson disease. Neurology 2005;64:1958–1960.
39. Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. Neurology 1992;42:1142–1146.
40. Passali D, Mezzedimi C, Passali GC, Nuti D, Bellussi L. The role of rhinomanometry, acoustic rhinometry, and mucociliary transport time in the assessment of nasal patency. Ear Nose Throat J 2000;79:397–400.
41. Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin' sticks': olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. Chem Senses 1997;22:39–52.
42. Hummel T, Kobal G, Gudziol H. kay-Sim A. Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. Eur Arch Otorhinolaryngol 2007;264:237–243.
43. Boesveldt S, De Muinck Keizer RJ, Knol DL, Wolters EC, Berendse HW. Extended testing across, not within, tasks raises diagnostic accuracy of smell testing in Parkinson's disease. Mov Disord 2009;24:85–90.
44. Savic I, Gulyas B, Larsson M, Roland P. Olfactory functions are mediated by parallel and hierarchical processing. Neuron 2000;26:735–745.
45. Blackinton JG, Anvret A, Beilina A, Olson L, Cookson MR, Galter D. Expression of PINK1 mRNA in human and rodent brain and in Parkinson's disease. Brain Res 2007;1184:10–16.
46. Gandhi S, Muqit MM, Stanyer L, et al. PINK1 protein in normal human brain and Parkinson's disease. Brain 2006;129:1720–1731.

Supplementary Table

Published data and present results on olfactory abilities in monogenic parkinsonisms.

Ref.	Patients / subjects	OT	OD	OI	Controls
[24]	*SNCA* A-Het	NT	NT	3/3	PND
[25]	*SNCA* A-Het	2/7	NT	2/7	PND
[24]	*LRRK2* A-Het	NT	NT	2/2*	PND
[47]	*LRRK2* A-Het	NT	NT	3/6	PND
[48]	*LRRK2* A-Het	NT	NT	5/5*	PND
[49]	*LRRK2* A-Het	NT	NT	mean (n=4) comparable to controls**	PND
[26]	*LRRK2* A-Het	NT	NT	11/13	PND
[50]	*LRRK2* A-Het	NT	NT	1/1	PND
[23]	*LRRK2* A-Het	NT	0/1	1/1	MCI
[28]	*LRRK2* A-Het	NT	NT	mean (n=21) significantly < than controls**	MCI
[27]	*LRRK2* A-Het	NT	NT	22/43	PND
[28]	*LRRK2* H-Het	NT	NT	0/3	MCI
[24]	Park3	NT	NT	1/1	PND
[23]	*Parkin* Hom	NT	3/5	0/5	MCI
[22]	*Parkin* Hom	NT	NT	mean (n=17) comparable to controls**	MCI
[23]	*DJ-1* Hom	NT	1/1	0/1	MCI
[35]	*PINK1* Hom	NT	NT	1/1	MCI
present study	*PINK1* Hom	3/7	5/7	7/7	MCI
present study	*PINK1* A-Het	5/6	6/6	4/6	MCI
present study	*PINK1* H-Het	7/12	7/12	0/12	MCI

For each olfactory task, number of patients with abnormal scores / total number of tested patients are reported. *patients defined as microsmic or anosmic, but some

reported values are considered normal in other reports; **individual values not available. A-Het, affected heterozygotes; H-Het, healthy heterozygotes; Hom, Homozygotes; MCI, matched controls internal to the study; NT, not tested; PND, published normative data.

Supplementary references

47 Berg D, Schweitzer K, Leitner P, et al. Type and frequency of mutations in the LRRK2 gene in familial and sporadic Parkinson's disease*. Brain 2005;128:3000-3011.

48 Hernandez DG, Paisan-Ruiz C, Inerney-Leo A, et al. Clinical and positron emission tomography of Parkinson's disease caused by LRRK2. Ann Neurol 2005;57:453-456.

49 Khan NL, Jain S, Lynch JM, et al. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. Brain 2005;128:2786-2796.

50 Goldstein DS, Imrich R, Peckham E, et al. Neurocirculatory and nigrostriatal abnormalities in Parkinson disease from LRRK2 mutation. Neurology 2007;69:1580-1584.