Non-motor symptoms in Parkinson's disease

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Is olfactory impairment in Parkinson disease related to phenotypic or genotypic characteristics?

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Abstract

Objective: To evaluate the relation between olfactory impairment (OI) and other impairment domains in Parkinson disease (PD) and the characteristics of OI in patients with certain genotypic characteristics.

Methods: In 295 nondemented patients with PD and 150 controls with a similar overall age and sex distribution, olfactory function was evaluated with the identification (ID) and discrimination (DIS) tests of the Sniffin' Sticks. In patients, demographic and clinical characteristics were evaluated, and genetic analyses were performed.

Results: Of all patients, 61% had an impaired ID and 43% had an impaired DIS. No significant correlations >0.4 were found between olfactory scores and other demographic or clinical variables. Age and sex accounted for the 22% explained variance of the ID score regression model, whereas age, sex, and disease duration accounted for the 15% explained variance of the DIS score regression model. *Parkin* and *DJ-1* mutation carriers (homozygous or heterozygous compound, n=6) had normal ID scores. *APOEε*2 or *APOEε*4 carriers had no significantly different olfactory scores than noncarriers. The allele distribution of the alpha-synuclein (*SNCA*)-REP1 polymorphism in groups with an impaired or normal ID or DIS was comparable.

Conclusions: Olfactory impairment (OI) in Parkinson disease (PD) may be unrelated to other impairment domains of the disease, which may indicate that olfaction is an independent feature of PD. *Parkin* and *DJ-1* mutation carriers had normal identification scores but the number of mutation carriers is too small to draw conclusions. The *APOE* genotype (*APOEε*2 or *APOEε*4 alleles) and *SNCA*-REP1 polymorphism do not seem to influence olfaction in PD.

Introduction

Olfactory impairment (OI) is one of the nonmotor features of Parkinson disease (PD), which may also include cognitive impairment, autonomic dysfunction, depression, nighttime sleep problems, daytime sleepiness, and psychiatric complications.[1] In PD, OI is common and may consist of impairments in odor detection, identification (ID), or discrimination (DIS).[2,3] OI may occur early in the disease[3] and even antedate the onset of motor symptoms[4], which is in line with pathological findings in PD showing that neurodegenerative changes may start in the lower brainstem and olfactory bulb, and extend gradually onto the rostral brainstem and cerebral cortex.[5]
In patients with PD, no associations were found between OI and cognitive impairment.[6,7] To our knowledge, no other nonmotor domains have been evaluated with respect to their relation with OI. Furthermore, only two genotype-phenotype studies evaluated olfaction in PD mutation carriers and found that ID scores of *Parkin* and *LRRK2* mutation carriers and controls were comparable.[8,9] Therefore, the aims of this study were to assess the relations between OI and other impairment domains in PD and to evaluate characteristics of OI in patients with certain genotypic characteristics.

Methods

Design

The study is part of the "PROfiling PARKinson's disease" (PROPARK) study, a longitudinal cohort study of patients with PD (n=420), who are profiled on phenotype, genotype, disability, and global outcomes of health, using valid and reliable assessment instruments for PD. In patients from this cohort with their annual appointment between November 2005 and August 2006 (n=337) olfactory tests were administered.

Participants

All patients fulfilled the United Kingdom Parkinson's Disease Society Brain Bank criteria for idiopathic PD.[10] Recruitment of patients was based on age at onset (AAO) and disease duration, which are important determinants of disease course in PD.[11]

The recruitment procedure has been described elsewhere.[12] For this particular study, patients with Mini Mental State Examination (MMSE) scores < 24 were excluded. No other selection criteria were applied. Most patients were assessed at the Leiden University Medical Center (LUMC). To avoid bias towards recruiting less severely affected patients, patients who were unable to come to the hospital were assessed at home. Controls (n=150) were volunteers recruited among employees and partners of patients from the outpatient clinics of the Departments of Neurology of the LUMC (n=80) and the VU University Medical Center (VUMC; n=70). Controls had no history of major olfactory or neurological disorders and were selected to match the overall age and sex distribution of the patients. Control characteristics have been published elsewhere.[13] This study was approved by the medical ethical committees of the LUMC and VUMC and all participants gave informed consent.

Measurement instruments

Olfactory assessment

For the Sniffin' Sticks ID test[14], 16 odorants in suprathreshold intensity were presented, in a multiple-forced choice format with four descriptions (written and verbal). Each stick was held approximately 2 cm in front of the nostrils for 2-3 seconds, with an interval of 20-30 seconds between each stick. For the Sniffin' Sticks DIS test[14], subjects were blindfolded and presented with 16 odor-triplets, with an interval of 30 seconds between each triplet. Each triplet consisted of two identical and one deviant odorant. Subjects were asked to select the odd odor out of three odorants presented, without the need to recognize or name the odors.

No participants who had a cold at the time of the assessment which may interfere with olfactory function were tested. The tests were administered birhinally in a well-ventilated room to avoid any background smell interfering with the test odors. In both tests, olfactory scores were defined as the number of correct responses (0-16). Both olfactory tests have been proven to be reliable and valid in controls.[14] To evaluate ID or DIS impairment in patients, Sniffin' Sticks cut-off points for age and sex groups based on control values, were used as described previously.[13]

PROPARK

Within PROPARK, all patients received standardized assessments, including evaluation of demographic and clinical characteristics, family history of PD, and medication use. Measurement instruments for the clinical PD domains were derived from a prior project (SCales for Outcomes in PArkinson's disease: SCOPA).[15]

For the current study, data obtained for disease severity (Hoehn & Yahr (H&Y))[16], motor function (SPES/SCOPA-motor, range 0-42)[17], cognition (SCOPA-COG, range 0-43)[18], autonomic function (SCOPA-AUT, range 0-69)[19], depressive symptoms (Beck Depression Inventory (BDI), range 0-63)[20], nighttime sleep (SCOPA-SLEEP NS, range 0-15) and daytime sleepiness (SCOPA-SLEEP DS, range 0-18)[21], and psychiatric complications (first six items of the SCOPA-PC, range 0-18)[22] were used. Except for the SCOPA-COG, higher scores indicate more severe impairment. Motor phenotype (tremor-dominant, postural instability gait difficulty (PIGD), or indeterminate) was determined for every patient with an earlier described method.[23]

Instruments were either self-administered (SCOPA-AUT, BDI, SCOPA-SLEEP) or administered by trained research associates (H&Y, SPES/SCOPA-motor, SCOPA-COG, SCOPA-PC). For reasons of comparability, all patients who used levodopa or a dopamine-agonist and experienced motor fluctuations, were assessed during 'on'-state. For each patient, a total levodopa equivalent (LDE) for the dose of levodopa and dopamine agonists was calculated.[24]

Genetic testing

Genomic DNA was isolated from peripheral blood using standard procedures.

Mutation screening

All patients were screened for mutations in exons 19, 31, 35, 38, 41 and 48 of the *LRRK2* gene, whereas only patients with an AAO ≤ 50 years (early onset PD (EOPD)) were screened for mutations in *Parkin, DJ-1,* and *PINK1* by direct sequencing of all exons. Additionally, patients with EOPD were screened for the alpha-synuclein (*SNCA*) A30P mutation and analyzed for genomic rearrangements of *SNCA, Parkin, DJ-1,* and *PINK1* genes.

Apolipoprotein E (APOE) genotyping

For *APOE* allelic discrimination, two non-synonymous coding single nucleotide polymorphisms (SNPs), rs429358 (R130C) and rs7412 (R176C), were genotyped. A validated TaqMan assay was used for detection of these SNPs (Applied Biosystems, Foster City, CA). For double heterozygotes a direct PCR-based restricted fragment length polymorphism method was used.[25]

SNCA genotyping

PCR was performed with 20 ng of DNA and the following conditions: initial denaturation of 95°C for 12 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. The final extension was at 72°C for 45 minutes. The sequence of PCR primers are: fluorescent-labeled Forward, 5′-CCTGGCATATTTGATTGCAA-3′, and reverse, 5′-GACTGGCCCAAGATTAACCA-3′. Two µl of 50X diluted PCR product was mixed with 10 µl of the following mixture, which was prepared with 10 µl of 500 Liz size standard (Applied Biosystems, Foster City, CA) in 1000 µl of formamide (Applied Biosystems). The mixture consisting of diluted PCR product and size standard-formamide was denaturated at 95°C for 5 minutes and cooled on ice for 10 minutes. Fluorescent labeled PCR fragments were resolved by the capillary electrophoresis on an ABI 3730 and allelic sizes assessed using GeneMapper® software version 4.0 provided by Applied Biosystems.

Statistical Analysis

If 25% or more of the data from a questionnaire or scale was missing, data from this scale for this patient were excluded from statistical analyses. If, for a particular patient, less then 25% of the items of a scale were missing, missing data were imputed by the mean value of the non-missing items. Differences between groups were analyzed with χ^2 tests, student's T-tests for independent samples, or analysis of covariance. Correlation coefficients were used to assess relations between ID or DIS scores and other demographic and clinical variables. Multiple forward linear regression analyses

were used to explore the contribution of different variables to the ID and DIS score. A p-value < 0.05 was considered significant. All analyses were performed with Statistical Package for the Social Sciences 14.0 Software (SPSS 14.0).

Results

Of the 337 patients, four patients were excluded due to too many missing values on both olfactory tests. Furthermore, 35 patients had MMSE scores < 24 and three patients had missing MMSE scores, and were also excluded. In total, 295 patients (65% men) with a mean (SD) age of 60.2 (10.6) years participated (table 1). Two patients had too many missing values on the ID test and were therefore excluded from analyses for that particular test. The percentages of patients with too many missing values on other variables and scales ranged from 0% (age, sex, AAO, disease duration, SCOPA-AUT, SCOPA-SLEEP, SPES/SCOPA-motor) to 5% (smoking status).

Table 1. Characteristics of patients with Parkinson disease

Characteristics	Patients
No. of patients	295
Sex, m/f (% men)	192/103 (65%)
Age, mean (SD), y	60.2 (10.6)
Disease duration, mean (SD), y	11.8 (6.3)
Age at onset, mean (SD), y	48.4 (11.2)
Hoehn and Yahr stage, %	
1/2/3/4/5/missing	4/42/37/14/2/1
Motor phenotype, %	
Tremor-dominant	37
Postural instability gait difficulty	50
Indeterminate	12
Missing	1
Total levodopa dosage equivalent, mean (SD), mg/d	683.5 (513.5)
Levodopa therapy, no. of patients (%)	214 (73%)
Dopamine-agonists therapy, no. of patients (%)	211 (72%)

Olfaction in patients

Patients had a mean (SD) ID score of 7.6 (3.0), and a mean (SD) DIS score of 8.3 (2.6). Women had higher mean scores than men on both tests (ID: 8.7 vs 7.1, p<0.001; DIS: 9.2 vs 7.9, p<0.001). Current smokers (n=19) were younger than nonsmokers (n=260) (p=0.001). After age correction, olfactory scores of smokers and nonsmokers were comparable (ID: p=0.350; DIS: p=0.095).

Patients scored lower than controls on both olfactory tests (both p-values <0.001), which was also found when analyzing scores of women and men separately (all p-values <0.001). Overall, 293 patients had valid scores on both olfactory tests, of which 27% had no OI (n=78, 42% men), 43% had impaired ID or impaired DIS (n=126, 70% men), and 30% had both impaired ID and DIS (n=89, 78% men).

Subgroup evaluations

Of 150 controls, 10 (7%) had an impaired ID, whereas of 293 patients with valid scores on the ID test, 178 patients (61%) had an impaired ID (p<0.001). These patients were significantly more often men, older, had a significantly older AAO, more severe PD as measured by H&Y, more motor, cognitive, and psychiatric problems, and significantly more daytime sleepiness, than patients with normal ID (table 2).

Of 150 controls, 6 (4%) had an impaired DIS, whereas of 295 patients with valid scores on the DIS test, 128 patients (43%) had an impaired DIS (p<0.001). Patients with an impaired DIS were more often men (p=0.004), were younger (p<0.001), and had a younger AAO (p<0.001) than patients with a normal DIS.

Patients with a tremor-dominant phenotype (n=110) were younger than patients with a PIGD phenotype (n=148) (p<0.001), but had comparable olfactory scores after correction for age (ID: p=0.646; DIS: p=0.726), or when analyzing women and men separately (women ID: p=0.134; women DIS: p=0.722; men ID: p=0.357; men DIS: p=0.908).

Table 2. Characteristics of patients with Parkinson disease with impaired and normal ID

Characteristics	Impaired ID	Normal ID	p-value
No. of patients	178	115	-
Age, mean (SD), y	62.6 (10.1)	56.3 (10.1)	<0.001[1]
Sex, m/f (% men)	133/45 (75%)	57/58 (50%)	<0.001[2]
Disease duration, mean (SD), y	12.0 (6.1)	11.2 (6.1)	0.321[1]
AAO, mean (SD), y	50.7 (11.1)	45.0 (10.5)	<0.001[1]
Hoehn and Yahr stage, %*			
1/2/3/4/5/missing	2/38/41/16/2/2	7/50/30/11/1/1	0.030[2]
Total LDE, mean (SD), mg/d	713.4 (474.3)	637.9 (570.9)	0.225[1]
SPES/SCOPA-motor score, mean (SD)	15.2 (5.5)	13.1 (5.3)	0.002[1]
SCOPA-COG score, mean (SD)	27.0 (5.9)	29.6 (5.3)	<0.001[1]
SCOPA-AUT score, mean (SD)	18.5 (8.2)	16.9 (8.2)	0.088[1]
BDI score, mean (SD)	9.5 (6.2)	10.0 (6.6)	0.482[1]
SCOPA-SLEEP NS score, mean (SD)	4.7 (3.6)	4.6 (3.3)	0.824[1]
SCOPA-SLEEP DS score, mean (SD)	5.4 (4.0)	4.3 (3.4)	0.019[1]
SCOPA-PC score, mean (SD)	2.4 (1.9)	1.8 (1.6)	0.004[1]

[1]: student's T-test for independent samples; [2]: χ^2 test

*: sum of percentages does not equal 100 due to rounding off

ID: identification; AAO: age at onset; LDE: levodopa dosage equivalent; BDI: Beck Depression Inventory; NS: nighttime sleep; DS: daytime sleepiness; SCOPA-PC: SCOPA-Psychiatric Complications

Determinants of ID and DIS scores

No significant moderate or strong correlations (r>0.4) were found between ID and DIS scores and other demographic or clinical variables. Multiple regression analysis revealed that age (15%) and gender (7%) accounted for the 22% explained variance of the ID score (total regression model; p<0.001) where lower age and female gender were associated with higher ID scores. Age (6%), gender (5%), and disease duration (4%) together explained 15% of the variance of the DIS score (total regression model; p<0.001) where lower age, female gender and shorter disease duration were associated with higher DIS scores (table 3).

Table 3. Determinants of ID and DIS scores in patients with Parkinson disease

	Variable[1]	R square	Standardized β
ID score	Age	0.15	-0.378
	Sex	0.07	-0.263
	Total	0.22	-
DIS score	Age	0.06	-0.203
	Sex	0.05	-0.243
	Disease duration	0.04	-0.180
	Total	0.15	-

[1]: multiple forward linear regression analysis was used with the variables age, sex, disease duration, total levodopa dosage equivalent, Hoehn & Yahr stage, cognitive functioning, autonomic functioning, depressive symptoms, nighttime sleep, daytime sleepiness, psychiatric complications. Variables are ordered in the table as they appeared in the model
ID: identification; DIS: discrimination

Olfaction in relation to genotypic characteristics

In total, 14 patients refused to donate blood and in 13 patients collection of DNA was not possible during the assessment. Furthermore, genotyping of the *SNCA*-REP1 polymorphism failed in 21 patients. Therefore, 268 patients were screened for *LRRK2* mutations and genotyped for *APOE* polymorphisms, whereas the *SNCA*-REP1 polymorphism was genotyped in 247 patients. The *SNCA* A30P mutation and *Parkin, PINK1,* and *DJ-1* mutations were screened in 159 patients with EOPD.

Mutation carriers

One patient had a heterozygous mutation in the *LRKK2* gene. In total, six patients had homozygous or compound heterozygous mutations in *Parkin* (n=5) or *DJ-1* (n=1). No patients had A30P mutations in the *SNCA* gene or compound heterozygous or homozygous mutations in the *PINK1* gene. The *LRRK2* mutation carrier had impaired ID but normal DIS. The *Parkin* mutation carriers had either normal olfactory scores (n=2) or normal ID but impaired DIS (n=3). The *DJ-1* mutation carrier had normal ID but impaired DIS.

APOE genotype and olfaction

Of 268 patients in whom *APOE* genotype was determined, 76 patients carried one (n=71) or two (n=5) *APOEε4* allele(s). There were no differences in age (p=0.063), sex (p=0.243), or olfactory scores (ID: p=0.106; DIS: p=0.387) between *APOEε4*-carriers and noncarriers. Furthermore, 42 patients carried an *APOEε2* allele. There were no differences in age (p=0.773), sex (p=0.205), or olfactory scores (ID: p=0.993; DIS: p=0.215) between *APOEε2*-carriers and noncarriers.

SNCA-REP1 polymorphism and olfaction

The *SNCA*-REP1 genotype was determined in 247 patients. Four alleles (266, 268, 270 and 272) of this polymorphism were observed in our population. Since only one copy of the 272-allele was present, it was excluded from the analyses. The allele distribution in groups with an impaired or normal ID (p=0.617) or DIS (p=0.167) was comparable.

Discussion

The aims of this study were to assess the relations between OI and other impairment domains in PD and to evaluate characteristics of OI in patients with certain genotypic characteristics. In line with others, we found lower olfactory scores in men and older patients, and no influence of smoking status.[26-29] Olfactory scores were comparable between patients with a tremor-dominant and PIGD phenotype, contrary to results of others.[26] In our sample of nondemented patients with PD, OI occurred in a large proportion of patients, with ID being more frequently impaired (61%) than DIS (43%), which was also found in a previous study with a largely overlapping population.[30] Most other studies, however, reported higher percentages of impaired patients.[2,29] Differences between our results and results of others could be due to the use of different olfactory tests or differences in sample characteristics. Most other studies used the University of Pennsylvania Smell Identification Test (UPSIT)[31], or an abridged version (B-SIT), as measure of olfaction.[7,26,27,29] The UPSIT is a 40-item self-administered standardized ID test. Differences between our results

obtained with the Sniffin' Sticks ID test and results of studies using the UPSIT or B-SIT could be due to several reasons. First, the UPSIT is a self-administered test, whereas the Sniffin' Sticks is administered by a trained assessor. Second, the original UPSIT has 40 items compared to 16 items in the Sniffin' Sticks ID test. Finally, there are differences in the odors used in the American UPSIT and the European Sniffin' Sticks.

In our study a relatively high percentage of patients have normal olfaction. Concerning neuropathology in PD, evidence has been presented for a sequential involvement of different regions of the central nervous system.[32] Braak stage I reflects involvement of the olfactory bulb, the anterior olfactory nucleus, and the dorsal motor nucleus of the vagus nerve.[32] A longitudinal study in patients with PD showed that in some patients olfactory function improved over time.[33] Furthermore, a significant improvement of DIS was noted in patients with PD treated with subthalamic deep brain stimulation.[34] These findings suggest that OI cannot be accounted for by cell loss only and may indicate a role for other mechanisms like complex adjustments in neuronal activities and network interactions.[35] In view of the large percentage of patients with normal olfaction, our findings apparently indicate a differential vulnerability of the olfactory circuitry to the different disease mechanisms that may operate in PD.

Patients with impaired ID had more problems on several impairment domains than patients with normal ID. However, impairment domain scores were comparable in patients with impaired and normal DIS. Furthermore, no significant moderate correlations between olfaction and impairment domains existed, and none of the impairment domains remained in the olfactory scores regression models and thus did not contribute to the explained variance. This may indicate that olfaction is not clearly related to other PD features and apparently, OI, like tremor[36], may behave as an independent feature of the disease. The lack of relation between OI and disease severity has been described by others[37], whereas relations with other nonmotor symptoms, except for cognition[6,7], have not been evaluated before. The lack of relations found between OI and other early nonmotor symptoms of PD could be

due to the long mean disease duration of our cohort (12 years). To reliably evaluate the relation between OI and other early nonmotor symptoms, an incident patient cohort would be more appropriate.

Our study shows that homozygous or compound heterozygous *Parkin* and *DJ-1* mutation carriers had normal ID, whereas the heterozygous *LRRK2* mutation carrier had impaired ID. With respect to *Parkin* and *DJ-1*, only homozygous or compound heterozygous carriers were considered mutation carriers and not single heterozygous carriers; both genes are autosomal recessive and therefore two mutations are necessary to be causative for the disease.

Three out of five mutation carriers had an impaired DIS. Our cohort did not contain *PINK1* (homozygous or compound heterozygous) or *SNCA* mutation carriers. Hitherto, the only studies evaluating olfaction in mutation carriers have been evaluating ID in homozygous and heterozygous (single and compound) *Parkin* mutation carriers and in *LRRK2* mutation carriers.[8,9] Patients in these studies also had normal ID[8,9], in accordance with our findings in *Parkin* mutation carriers. Our findings on ID in *Parkin* mutation carriers corroborate those of others, and one could speculate that specific genotypic characteristics account for certain olfactory characteristics. In this case, olfactory testing could become useful in separating, for example, Lewy body from non Lewy body PD. However, one has to take into account that because of the few mutation carriers, conclusions cannot be drawn at this stage.

The *APOEε2* and *APOEε4* allele are both described as risk factors for PD.[38,39] The *APOEε4* allele also is a risk factor for Alzheimer disease, a disease that is associated with OI.[40] In the general population, nondemented *APOEε4*-carriers had worse ID than noncarriers[40], which could indicate that the presence of an *APOEε4* allele by itself is associated with OI. The results of our study show that in PD neither the ε4 nor the ε2 allele seems to contribute to OI. Finally, there was no significant effect from the different alleles of the *SNCA*-REP1 polymorphism on olfaction. A limitation of our study was that the genetic analyses were not performed in all patients. Patients refused to donate blood, or DNA collection during the assessment was not possible, or the genotyping of the *SNCA*-REP1 polymorphism failed. Furthermore, because

Parkin, DJ-1, PINK1, and *SNCA* mutations usually occur in patients with EOPD, screening for these mutations was only done in these patients. The results might have been different if genetic screening would have been done in all patients. This study shows that OI in PD may be unrelated to other impairment domains of the disease. Considering genotypic characteristics, *Parkin* and *DJ-1* mutation carriers had normal ID scores whereas the *APOE* genotype (*APOEε*2 or *APOEε*4 alleles) and *SNCA*-REP1 polymorphism do not seem to influence olfaction in PD.

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