Olfactory testing combined with dopamine transporter imaging as a method to detect prodromal Parkinson’s disease

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Submitted
Abstract

Olfactory dysfunction is an early and common symptom in Parkinson’s disease (PD). Previously, we demonstrated that idiopathic olfactory dysfunction in first-degree relatives of PD patients is associated with an increased risk of developing PD. The aim of the present study was to determine the value of combined olfactory testing and SPECT scanning in predicting future PD in the same population of relatives over a five-year period.

In a cohort of 361 non-parkinsonian, non-demented first-degree relatives of PD patients, a combination of olfactory processing tasks was used to select groups of hyposmic (n=40) and normosmic (n=38) individuals for a five year clinical follow-up evaluation and sequential SPECT scanning, using a dopamine transporter ligand to assess nigrostriatal dopaminergic function at baseline and five years from baseline. A validated questionnaire, sensitive to the presence of parkinsonism, was used in the follow-up of the remaining 283 relatives.

Five years from baseline, 5 out of the 40 hyposmic relatives fulfilled clinical diagnostic criteria for PD. None of the other 349 relatives available for follow-up developed PD. All hyposmic individuals developing PD had an abnormal baseline SPECT scan.

In conclusion, idiopathic hyposmia in first-degree relatives of PD patients is associated with an increased risk of developing clinical PD of 12.5% over a five-year period. The present data suggest that a two-step approach using olfactory testing followed by SPECT scanning in hyposmic individuals has very high sensitivity and specificity in detecting PD. The usefulness of this two-step approach needs to be confirmed in larger populations.
INTRODUCTION

An important pathological characteristic of PD is the degeneration of dopaminergic neurons located in the substantia nigra, pars compacta, and their projections to the striatal regions. The extent of degeneration of the nigrostriatal dopaminergic system in PD can be visualized in vivo by means of positron emission tomography (PET) or single-photon emission computed tomography (SPECT)\(^1\), using radioligands for the dopamine transporter such as \(^{123}\text{I-}\) labelled \(\beta\)-CIT (2\(\beta\)-carbomethoxy-3\(\beta\)-tropane)\(^2\) or \(^{11}\text{C-labelled PE2I (2}\beta\text{-carbomethoxy-3}\beta\text{-}(4’-methylphenyl) nortropane)}\(^3\).

Results from in vivo PET and SPECT imaging studies in PD patients suggest that the onset of dopaminergic neuronal loss antedates the clinical diagnosis of PD by four to six years\(^4\)\(^-\)\(^7\). Subclinical reductions in dopamine transporter binding have indeed been detected in at-risk individuals that later developed the classical motor signs of PD\(^7\)\(^-\)\(^8\). However, taking into account radiation exposure and cost-effectiveness, evaluating the dopamine transporter binding capacity in the general population by means of this technique would not be a suitable screening strategy for PD. It is therefore important to identify risk factors for PD that can be used in conjunction with nuclear imaging techniques as elements of an early diagnostic procedure to identify participants in the prodromal phase of PD.

Over the past few years a number of independent groups have demonstrated that hyposmia is a risk factor for the development of PD. In 2004, we reported that idiopathic olfactory dysfunction is associated with an increased risk of developing PD of at least 10\(^%\)\(^9\). Subsequently, Haehner and co-workers found that 7\% of individuals with olfactory loss developed clinical PD\(^10\). Recently, the Honolulu-Asia Aging Study\(^11\) confirmed these observations in a non-selected population; the odds ratio for the development of PD associated with hyposmia was 5.2. Although there can be no doubt that hyposmia is a risk factor for PD in both selected and non-selected population, the absolute risk of future PD associated with olfactory dysfunction is not very high. Most likely, this can be explained by a lack of specificity of hyposmia for PD: olfactory impairments can occur in many other conditions some of which are quite prevalent such as viral infections of the nasal cavity and traumatic brain injury. A two-step approach, combining olfactory testing and dopamine transporter SPECT scanning, might significantly increase specificity.

Two year follow-up data using combined olfactory testing and SPECT scanning in a cohort of first-degree relatives of PD patients were reported previously\(^9\). In this same study, the hyposmic relatives that remained non-parkinsonian, as a group, showed an increased rate of loss of dopamine transporter binding over the two-year period, possibly indicative of incipient degeneration of the nigrostriatal dopaminergic system. Five year follow-up of this prospective study is now completed.
The aim of this study was to determine the value of combined olfactory testing and SPECT scanning in predicting future PD in first degree relatives of PD patients over a five year period. An additional goal was to determine whether the incipient degeneration of the nigrostriatal dopaminergic projection system that was observed two years from baseline in hyposmic, but non-parkinsonian relatives would progress over time.

**Materials and Methods**

*Study population / participants*

The present study involved 361 first-degree relatives (285 children, 73 siblings and 3 parents) of patients with sporadic PD. Participants were recruited partly from the general population and partly from family members of patients at the outpatient clinic for movement disorders of the VU University Medical Center. As described previously, relatives were included when they fulfilled the following criteria: (1) clinical diagnosis of PD in the affected relative obtained by a neurologist or established retrospectively using information obtained from the unaffected relatives; (2) absence of a history of other (neuropsychiatric) disorders or conditions known to influence olfactory function; (3) no medication that might influence dopamine transporter binding and/or olfactory function; (4) absence of parkinsonism as defined by the United Kingdom Parkinson’s Disease Society Brain Bank (UK-PDSBB) criteria; (5) Unified Parkinson’s Disease Rating Scale (UPDRS) motor score < 5; (6) Cambridge examination for mental disorders (CAMCOG) orientation and memory section score > 26. All participants gave written informed consent; the protocol of the study was approved by the Health Council of The Netherlands and the local medical ethical committees of both the VU University Medical Center and the Academic Medical Center.

*Study design*

At baseline, all 361 participants were submitted to a combination of olfactory processing tasks (see figure 1). The average Z-score over the three olfactory tests was chosen as a measure of olfactory function. Participants were selected for SPECT scanning from five consecutive groups of 70 to 80 participants to limit the interval between olfactory testing and baseline SPECT scanning. To reduce the effect of age- and gender-related differences in olfactory performance, a rank order based on average Z-scores was created for men and women separately in three groups of participants aged 50-59 years and in two groups of individuals aged 60-75 years. In each group, those individuals with the 10% lowest average Z-scores (with the
Figure 1. Flow-chart illustrating the overall design of the study.

361 relatives of PD patients

olfactory tests

38 normosmic relatives

SPECT scanning

1 lost to follow-up *

n=2 ***

35 normosmic relatives

SPECT scanning ****

clinical evaluation

questionnaire

n=18

clinical evaluation

283 relatives

6 lost to follow-up **

n=1 ***

280 relatives

39 hyposmic relatives

SPECT scanning*****

clinical evaluation

* The lost relative passed away.

** Six lost to follow-up, five of whom passed away.

*** Two normosmic relatives and one hyposmic relative switched to the questionnaire group.

**** One relative did not have SPECT scanning because of SSRI use.

***** Three relatives did not have SPECT scanning for various reasons.
additional requirement that the performance on each of the olfactory tasks had to be below group average) were considered hyposmic. In this way, 40 hyposmic relatives (29 children, 11 siblings) were identified. Similarly, 38 relatives (31 children, 7 siblings) with the highest average Z-scores (and all olfactory scores above group average) were selected. The groups selected for SPECT scanning did not differ with regard to baseline demographics (see table 1).

Five years after baseline, 74 out of the 78 individuals that were scanned at baseline were available for follow-up (see figure 1). Three hyposmic relatives and one normosmic relative did not have a follow-up SPECT scan for various reasons. Of the 283 relatives not selected for baseline SPECT scanning, 280 completed a questionnaire sensitive to the presence of parkinsonism as part of the five-year follow-up evaluation (see figure 1). Relatives with possible parkinsonism according to the questionnaire were invited to the outpatient clinic for movement disorders of the VU University Medical Center for clinical evaluation.

### Olfactory processing tasks

Olfactory function was assessed, as described previously, by means of a combination of an odor detection, an odor discrimination and an odor identification task. The odor detection task was adapted from a task developed by Doty. The odor discrimination task used was developed at the University Medical Center Utrecht. A modified version of the 12-item Cross-Cultural Smell Identification Test (CC-SIT), adapted to the Dutch population, was used to assess odor identification.

### Clinical evaluation

Five years from baseline, clinical evaluation of all individuals in the two groups selected for [123I]-CIT SPECT scanning was carried out by a movement disorders specialist and included a screening neurological examination and a specific assessment to detect the presence of parkinsonism as defined by the UK-PDSBB criteria. Motor function was rated by means of the motor section of the UPDRS. A UPDRS motor score of five or more was considered as a sign of motor dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>All subjects (n=361)</th>
<th>Normosmic relatives (n=38)</th>
<th>Hyposmic relatives (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>157/204</td>
<td>17/21</td>
<td>19/21</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>58.7 ± 6.6</td>
<td>58.5 ± 6.6</td>
<td>59.2 ± 5.8</td>
</tr>
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</table>
not necessarily as part of a parkinsonian syndrome. Individuals that had developed clinical PD prior to the five year follow-up evaluation and were already using medication were tested off-medication, at least 12 hours after their evening dose.

A Dutch translation of a screening questionnaire for PD (for a description see\textsuperscript{9,17}) was administered to the 280 relatives not selected for SPECT scanning. This mail questionnaire comprises nine symptom questions. With a cut-off score of three or more positive responses, sensitivity and specificity to detect parkinsonism are 95\% and 89\%, respectively. An extra question was added to establish whether a physician had made a diagnosis of PD over the course of the follow-up period. Three or more positive responses to the screening questionnaire, as well as a positive response to the additional question were considered indicative of possible parkinsonism.

Individuals with possible parkinsonism according to the questionnaire were submitted to a structured clinical work-up, comprising a standard history taking and a neurological examination including the UPDRS motor score. A blinded movement disorders specialist, not involved in the baseline screening, performed the structured clinical work-up.

\textit{SPECT scanning}

SPECT studies were performed using a 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half-maximum resolution of approximately 6.5 mm, throughout the 20 cm field-of-view, according to a previously described method\textsuperscript{7}, using the well-validated dopamine transporter tracer \textsuperscript{123I}\(\beta\)-CIT [de Win et al., NPP 2005]. \textsuperscript{123I}\(\beta\)-CIT (specific activity > 185 MBq/nmol; radiochemical purity > 99\%) was injected intravenously at an approximate dose of 110 MBq, and imaging was performed 24 h after injection. Attenuation correction of all images was performed as described earlier\textsuperscript{18}. Compared to our previously published data\textsuperscript{7,9}, the image reconstruction method was updated, resulting in a 3D mode reconstruction (http://www.neurophysics.com). These 3D reconstructed images (baseline and 5-year follow-up images) were then randomly numbered and analyzed blinded to olfactory performance by a single observer. For quantification, a region-of-interest (ROI) analysis was performed using a previously described method\textsuperscript{7}. Analysis of bilateral striatal, bilateral putamen, and bilateral caudate \textsuperscript{123I}\(\beta\)-CIT binding for each baseline and follow-up scan was performed using a previously described method\textsuperscript{7}. Baseline SPECT scans of one hyposmic relative and three normosmic relatives, could not be analyzed quantitatively due to technical problems.

For both baseline and follow-up SPECT scans, group means of each binding parameter were calculated and compared by means of Student’s unpaired \textit{t} test. In
addition, for each baseline SPECT parameter, age-adjusted means and its 98% confidence interval were determined in the group of normosmic relatives. Individual baseline values of hyposmic relatives were considered abnormal if they fell outside the 98% confidence interval of the age-adjusted means of the group of normosmic individuals.

Linear regression analysis was used to compare the average rate of decline in $[^{123}\text{I}]\beta$-CIT binding ratios over the five year follow-up period between groups. The five year follow-up data were used as the dependent variable and group as an independent variable. By adding the baseline value as an independent variable, changes in $[^{123}\text{I}]\beta$-CIT binding ratios were corrected for the baseline value (autoregression analysis). Since age and gender did not differ between the groups (tested using univariate analysis of variance and a chi-square test, respectively), these factors were not used as independent variables.

**Results**

**Clinical evaluation**

Five years from baseline testing, five relatives (12.5% out of 40 baseline hyposmic relatives) had developed clinical PD as defined by the UK-PDSBB criteria. Initial clinical (motor) symptoms appeared 9 to 52 months (median 15 months) after baseline testing. Five years from baseline, “off”-medication UPDRS motor scores in these patients were 13, 16, 18, 29 and 52. Three out of these five relatives were using antiparkinsonian medication (dopamine-agonist and/or levodopa), and had a good clinical response. Of the other 349 relatives available for follow-up, none fulfilled UK-PDSBB criteria for a parkinsonian syndrome. This included the 280 relatives in the questionnaire group and the 69 non-parkinsonian relatives in the groups selected for SPECT scanning and clinical evaluation. In the questionnaire group, 18 relatives had three to seven (out of nine) positive responses to the screening questionnaire. Subsequent clinical neurological evaluation did not reveal a parkinsonian syndrome in any of these 18 relatives.

$[^{123}\text{I}]\beta$-CIT SPECT imaging

Mean SPECT binding ratios at baseline and five years from baseline for the hyposmic and normosmic groups are listed in table 2. Five years from baseline, mean bilateral whole striatal, caudate and putamen binding ratios were not significantly different between normosmic relatives and hyposmic relatives without parkinsonism. Of the hyposmic relatives with parkinsonism, all parameters were significantly different from the normosmic relatives and from the hyposmic relatives without
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Table 2. Specific to non-specific [¹²³I]β-CIT binding ratios (mean ± SD) at baseline and five year follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Five year follow-up</th>
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<tbody>
<tr>
<td></td>
<td>Normosmic relatives</td>
<td>Hydroptic relatives</td>
</tr>
<tr>
<td></td>
<td>n=35</td>
<td>n=39</td>
</tr>
<tr>
<td>Left striatum</td>
<td>7.79 ± 0.9</td>
<td>7.55 ± 2.2</td>
</tr>
<tr>
<td>Right striatum</td>
<td>7.77 ± 1.0</td>
<td>7.53 ± 2.1</td>
</tr>
<tr>
<td>Left putamen</td>
<td>5.94 ± 0.9</td>
<td>5.76 ± 1.8</td>
</tr>
<tr>
<td>Right putamen</td>
<td>5.90 ± 0.9</td>
<td>5.74 ± 1.7</td>
</tr>
<tr>
<td>Left caudate</td>
<td>8.87 ± 1.0</td>
<td>8.96 ± 2.7</td>
</tr>
<tr>
<td>Right caudate</td>
<td>8.91 ± 1.0</td>
<td>8.90 ± 2.6</td>
</tr>
</tbody>
</table>

parkinsonism. Compared to previously published data⁷,⁹, the updated 3D image reconstruction method (as described in the method section) resulted in comparable, but slightly higher absolute binding values.

At baseline, the five hyposmic relatives who later developed clinical PD (out of the 39 hyposmic relatives) showed at least one reduced [¹²³I]β-CIT binding ratio (i.e., measurements for the whole striatum, putamen or caudate nucleus) outside the 98% confidence interval of the age-adjusted means of the normosmic relatives; three relatives with all six binding ratios reduced, one relative with reduced right striatal and right putamen [¹²³I]β-CIT binding ratios and one relative with reduced left and right putamen [¹²³I]β-CIT binding ratios (left and right putamen [¹²³I]β-CIT binding ratios are illustrated in figure 2). A single normosmic relative had marginally reduced left and right striatal and putamen [¹²³I]β-CIT binding ratios at baseline, but did not develop clinical parkinsonism over the five year follow-up period.

Over the five year follow-up period, the average rate of decline in [¹²³I]β-CIT binding ratios was not significantly different between normosmic relatives and hyposmic relatives. Compared to the normosmic relatives, there was a trend towards a higher rate of decline (p=0.093) in left putamen binding in the hyposmic group. This trend disappeared when the five hyposmic relatives who had already developed clinical PD were excluded from the analysis. Figure 3 illustrates the individual values for the rates of change in left and right striatal [¹²³I]β-CIT binding, expressed as percentage change from baseline in [¹²³I]β-CIT binding ratios. No individual hyposmic or normosmic relative had a particularly rapid loss of binding.
Figure 2. Scatter plots of left (A) and right (B) putamen $[^{123}]\beta$-CIT binding ratios at baseline. Open circles = normosmic relatives (n=35); filled circles = hyposmic relatives who remained non-parkinsonian (n=34); filled triangles = hyposmic relatives who developed clinical parkinsonism (n=5); solid lines = the age-adjusted means and the 98% confidence interval of $[^{123}]\beta$-CIT binding values in the group of normosmic relatives.

Figure 3. Scatter plots of the rate of change in left (A) and right (B) striatal $[^{123}]\beta$-CIT binding ratios over the five year follow-up period. Open circles = normosmic relatives (n=31); filled circles = hyposmic relatives who remained non-parkinsonian (n=31); filled triangles = hyposmic relatives who developed clinical parkinsonism (n=4); solid lines = mean change in $[^{123}]\beta$-CIT binding values.
DISCUSSION

In this five year follow-up study in first-degree relatives of PD patients, idiopathic hyposmia at baseline was associated with an increased risk of subsequently developing clinical PD of 12.5%. Furthermore, all hyposmic individuals who had developed PD within five years from baseline, had abnormal striatal dopamine transporter binding at baseline. No evidence was found for a subclinical degeneration of the nigrostriatal dopaminergic system in non-parkinsonian hyposmic relatives.

A number of recent studies from several research groups have demonstrated that hyposmia is a risk factor for PD in both selected and non-selected populations. In the most recent study by Ross et al., the association between hyposmia and an increased risk for the development of PD was found only for the first four years of follow-up, not for the second period of four years. The results of the present study, combined with those of the two-year follow-up data from the same cohort, show that the risk of developing clinical PD in hyposmic relatives of PD patients is higher in the first two years from baseline testing than in the subsequent three years. In addition, all individuals who developed clinical PD had abnormal baseline striatal dopamine transporter binding, illustrating the presence of nigrostriatal degeneration in advance of clinical symptoms. This observation is in line with the results of nuclear imaging and postmortem studies, suggesting that the onset of nigral dopaminergic neuronal loss antedates the clinical diagnosis of PD by about four to six years. According to the pathological staging system put forward by Braak and colleagues, the neuropathological process may actually affect the olfactory bulb and related portions of the anterior olfactory nucleus long before the substantia nigra. This would suggest that the prodromal phase of PD might be substantially longer than estimated on the basis of the aforementioned imaging data. So far, the results presented here and the observations by Ross et al. do not seem to support this.

In the present cohort of 361 first degree relatives of PD patients, five individuals (12.5% of the 40 hyposmic relatives) developed PD. In the general population, the incidence rates for PD are 0.3 and 1.4 per 1000 person-years for individuals aged 55 to 65 years and 65 to 75 years, respectively. Taking into account the fact that the prevalence of PD is three-four times higher in first degree relatives of PD patients than in the general population, the expected incidence of new PD cases in our cohort would be approximately five, which is exactly the number we identified. Compared to previous studies, which demonstrated increased risks of developing clinical PD in the range of 2-7% for hyposmic individuals, the present observation of 12.5% is relatively high. Nevertheless, the absolute risk of developing clinical PD remains too low to consider olfactory testing as a single test to be used as a screening method to detect individuals at risk for PD. The results of the present study show that a two-
step approach of initial olfactory testing followed by dopamine transporter SPECT scanning in hyposmic individuals strongly increases specificity relative to using olfactory testing alone as an early diagnostic strategy to detect prodromal PD. At the same time, sensitivity seems to be maintained in this combined approach, although we should bear in mind that the number of cases developing PD in this study was too low to draw a very firm conclusion. In considering the application of a two-step olfactory testing – SPECT imaging approach to the general population for screening purposes, it is clear that this approach would require many SPECT scans in individuals not actually suffering from PD. As such, the two step olfactory testing – SPECT imaging approach would still need improvement to make it feasible for large scale screening. One way of doing so would be to expand the first screening step to include tests sensitive to other prodromal features of PD, such as REM sleep behaviour disorder, or genetic susceptibility factors.

Two years from baseline in this same cohort of first degree relatives of PD patients, non-parkinsonian hyposmic relatives showed an increased rate of decline in dopamine transporter binding compared to the normosmic relatives, possibly indicative of incipient degeneration of the nigrostriatal dopaminergic system. This difference in the rate of loss of dopamine transporter binding could not be confirmed in the present analysis after five years of follow-up. The longer five year interval between scans in the present study is more likely to yield data less susceptible to biological variation in dopamine transporter binding over time in a single individual. This type of variation may have influenced the SPECT data obtained two years from baseline. The average rate of change in striatal \([123]I\)\(\beta\)-CIT binding in the hyposmic relatives who had developed PD during the follow-up period of 5 years was 28.5\%, which is in line with the 6-13\% annual reduction reported previously for PD patients.

A limitation of the present study is that baseline SPECT scanning was performed in only 78 out of 361 individuals, possibly missing some individuals with a subclinical degeneration of the nigrostriatal system. However, given the length of the follow-up period, it is not very likely that an individual with an undetected subclinical degeneration of the dopaminergic system at baseline would not have developed clinical signs of PD five years later. These signs would then have been detected by means of the questionnaire with high sensitivity in detecting PD that was sent to all individuals that were not scanned. Another potential limitation of the present study is that we used a selected sample of relatives of PD patients, excluding participants with a history of other disorders or conditions known to influence olfactory. This might explain the high predictive value of 12.5\% of the current study compared to the value of 2\% found in an unselected population.

An important strength of the current study is its prospective, longitudinal design.
Another strength is the two-step approach, combining olfactory testing with SPECT scanning. This increased specificity in detecting individuals at risk for developing PD compared to olfactory testing alone. Furthermore, in spite of the long interval between baseline evaluation and follow-up, only very few individuals were lost to follow-up.

In conclusion, idiopathic hyposmia in first degree relatives of PD patients is associated with an increased risk of developing clinical PD within five years of 12.5%. A two-step approach using olfactory testing followed by SPECT scanning in hyposmic individuals appears to have very high sensitivity and specificity in detecting PD in its prodromal phase. The usefulness of this two-step approach needs to be confirmed in larger populations, possibly also including additional clinical or genetic risk factors in the initial screening step.
REFERENCES


