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ORIGINAL RESEARCH

Exploring white matter microstructure and olfaction dysfunction in early parkinson disease: diffusion MRI reveals new insight

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Abstract
Olfaction dysfunction is considered as a robust marker of prodromal Parkinson disease (PD). Measurement of olfaction function as a screening test is unsatisfactory due to long lead time interval and low specificity for detection of PD. Use of imaging markers might yield more accurate predictive values and provide bases for combined use of imaging and clinical markers for early PD. Diffusion MRI connectometry was conducted on 85 de novo PD patients in and 36 healthy controls to find: first, white matter tracts with significant difference in quantitative anisotropy between PD groups with various degrees of olfaction dysfunction and second, second fibers with correlation with University of Pennsylvania Smell Identification Test (UPSIT) score in each group using a multiple regression analysis considering age, sex, GDS and MoCA score. Local connectomes were determined in seven of all the possible comparisons, correcting for false discovery rate (FDR). PD patients with anosmia and normal olfaction had the highest number of fibers with decreased connectivity in left inferior longitudinal fasciculus, bilateral fornix, bilateral middle cerebellar peduncle (MCP), bilateral cingulum, bilateral corticospinal tract (CST) and body, genu and splenium of corpus callosum (CC) (*FDR = 0.0013*). In multiple regression analysis, connectivity in the body, genu and splenium of CC and bilateral fornix had significant negative correlation (*FDR between 0.019 and 0.083*), and bilateral cingulum and MCP had significant positive correlation (*FDR between 0.022 and 0.092*) with UPSIT score. White matter connectivity in healthy controls could not be predicted by UPSIT score using the same model. The results of this study provide compelling evidence that microstructural degenerative changes in these areas underlie the clinical phenotype of prodromal olfaction dysfunction in PD and that diffusion parameters of these areas might be able to serve as signature markers for early detection of PD. This is the first report that confirms a discriminative role for UPSIT score in identifying PD specific changes in white matter microstructure. Our results open a window to identify microstructural signatures of prodromal PD in white matter.

Keywords University of Pennsylvania Smell Identification Test (UPSIT) · Connectometry · Diffusion MRI · Anosmia · Microsomia · Early Parkinson disease

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Introduction

The overall prevalence of olfaction dysfunction in Parkinson Disease (PD) can reach up to 90% (Wang et al. 2011), making it the most common non-motor symptom (NMS) in

PD patients (Schapira et al. 2017). Olfaction dysfunction has proven to be an invariant marker of the underlying neurodegenerative process by many studies (Ross et al. 2008; Haehner et al. 2007; Berg et al. 2012). In particular, olfaction dysfunction has a high predictive value for imminent Parkinson's disease with a relative risk estimated to be 3.9 to 5.2-fold in population-based studies (Postuma and Berg 2016).

The degree of smell identification loss in PD is poorly associated with the severity of neural loss in cortical olfactory areas (Braak et al. 2004). Meanwhile, the spread of lewy body aggregations in olfactory bulb can more precisely predict olfaction loss in PD patients or even in asymptomatic individuals, as Lewy body deposition in olfactory bulb was shown to be more common in postmortem brain specimen of subjects with lower smell identification scores (Tredici et al. 2002; Wilson et al. 2011).

High sensitivity and simplicity of olfactory testing have made it a notable target to identify prodromal PD (Pellicano et al. 2007). Nonetheless, there are a number of discouraging features in the use of olfaction loss as a clinical marker of early PD, these include, uncertain lead-time between the onset of olfactory deficit and initiation of PD, which could be up to ten years, high prevalence of mild degrees of loss of olfaction in general population and the resulting low specificity of this marker for detection of PD (Mahlknecht et al. 2015). The combination of imaging markers with clinical features could yield higher predictive values for prodromal PD (Doty 1994; Ressler et al. 1994). One study linked white matter hyperintensities in FLAIR MRI sequence to the olfactory deficit in early PD (Ham et al. 2016). This model could well predict progression of cognitive decline and deterioration of semantic fluency on follow-up of PD patients.

The Parkinson's Disease Progression Marker Initiative (PPMI) is a landmark study to validate the probable biomarkers or risk factors in Parkinson disease (Marek et al. 2011). To test for the proposed additional predictive value of "smell deficit" and "imaging markers," it was hypothesized that there exist signature areas in which disrupted microstructure could predict the degree of olfactory impairment in early PD. First, regions with a different pattern of connectivity between PD patients with various degrees of smell loss were investigated. It was analyzed whether categorizations based on University of Pennsylvania Smell Identification Test (UPSIT) score yielded different neural substrates in diffusion MRI connectometry in PD patients. The full, 40-Item version of UPSIT was implemented in this database to identify olfaction dysfunction, and a multiple regression was employed to investigate the correlation of UPSIT score with white matter QA of fibers in early PD. It was then tested whether UPSIT score can predict similar areas in healthy controls enrolled in the PPMI database from the general population.

Materials and methods

Participants

Participants in this research were recruited from Parkinson's Progression Markers Initiative (PPMI, http://www.ppmi-info.org/). The study was approved by the institutional review board of all participating sites in the Europe, including Attikon University Hospital (Greece), Hospital Clinic de Barcelona and Hospital Universitario Donostia (Spain), Innsbruck University (Austria), Paracelsus-Elena Clinic Kassel/University of Marburg (Germany), Imperial College London (UK), Pitié-Salpêtrière Hospital (France), University of Salerno (Italy), and in the USA, including Emory University, Johns Hopkins University, University of Alabama at Birmingham, PD and Movement Disorders Center of Boca Raton, Boston University, Northwestern University, University of Cincinnati, Cleveland Clinic Foundation, Baylor College of Medicine, Institute for Neurodegenerative Disorders, Columbia University Medical Center, Beth Israel Medical Center, University of Pennsylvania, Oregon Health & Science University, University of Rochester, University of California at San Diego and University of California, San Francisco. Written informed consent was obtained from all the participants before study enrollment. The study was performed with relevant guidelines and regulations (Marek et al. 2011). The participants were tested and confirmed negative to any neurological disorder apart from PD. The participants' PD status was confirmed by Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS), and the loss of dopaminergic neurons was observed by DAT scans. Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) was applied in order to confirm PD status of enrolled participants. Subjects were only excluded if imaging failed specific quality control criteria.

Based on the initial quality control analysis performed by Dr Zhang and her colleagues (Zhang et al. 2016), we included 85 cases we PD from the total of 122 subject with DMRI data in baseline visit. The baseline DMRI data also consisted of 50 healthy controls, from which we included 36 subjects in the analyses. The case selection process was as follows:

Through visual inspection of fiber reconstruction results in DSI-Studio software, partial or total distortions in the shape and/or alignment of corpus callosum was detected in 32 subjects with PD and 12 subjects form the healthy cohort. This might be due to lesional alterations in patient's corpus callosum, corpus callosum agenesis, or due to problems with image acquisition. These subjects were therefore excluded from further analyses. Five other patients from the PD group were put aside to match for depression scale, REM sleep behavior disorder score, cognitive state, and/or age and sex.

Also two patients from the healthy control group had withdrawn from the PPMI study group by the time of analysis. Finally, a total 85 PD patients, and 36 healthy controls were recruited for between-group and multiple regression analyses on baseline available DMRI data from the PPMI project.

Odor identification was assessed using the University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al. 1984), and UPSIT raw scores were normalized for age and sex according to the method described by Jennings et al. (2014). We did not use cohort-specific norms, as described by Jennings, to categorize PD patients based on their UPSIT value. Instead, we used cutoff values of base-line olfaction for the same database from a 2016 study by Fullard and her colleagues (Fullard et al. 2016). Based on their normalized UPSIT score, PD patients were divided into five categories: PD patients with normosmia, normal olfaction, with UPSIT score of above 33, mild, moderate or severe macrosomia with the score between 30 and 33, 26–29 and 19–25 respectively and anosmia with scores lower or equal to 18 (The Parkinson Progression Marker Initiative (PPMI) 2011).

Participants in each category of the PD group (i.e. 18 patients with anosmia, 26 with severe macrosomia, 17 with moderate microsomia and 12 with mild microsomia and 12 PD patients with normal olfactory status) were matched in their age, sex, disease duration and education years, using One-way ANOVA between group statistics (Table 1). PD subjects were also comparable in motor severity (Hoehn and Yahr (H&Y) stage, Unified Parkinson's Disease Rating Scale part III (UPDRS-III)) and cognitive state (Montreal Cognitive Assessment (MOCA) score, Hopkins Verbal Learning Test (HVLT) Immediate Recall, Benton Judgment Of Line Orientation (JoLO), Semantic Fluency and Symbol Digit Modalities Scores), geriatric depression scale (GDS), and in REM sleep behavior disorder (RBD), using One-way Analysis Of Covariance (ANCOVA) with age, sex, and disease duration as covariates (Table 1). Healthy controls mainly had normal olfaction (19 subject with normal olfaction and 11 subjects with mild microsmia). Three patients had moderate microsmia, two had severe microsmia and only one was anosmic (Table 1).

Data acquisition

The data of this article was obtained from the Parkinson's Progression Markers Initiative (PPMI) database (http://www.ppmiinfo.org/data) (Marek et al. 2011). This

Table 1 Demographic and baseline clinical information of patients with Parkinson disease

Groups	Anosmia (n = 18)	Severe microsmia (n = 26)	Moderate microsmia (n = 17)	Mild microsmia (n = 12)	Normal olfaction (n = 12)	p-value**	Healthy controls (n = 36)
Age (mean ± sd)	58.7 ± 7.5	59.3 ± 9	57.4 ± 9.9	54.7 ± 8.8	53.4 ± 7.8	0.296	60.1 ± 10.6
Female/Male no	7/11	12/14	2/17	4/8	7/5	0.346	14/22
Handedness (L/R)	2/16	1/21	2/15	3/9	2/10	0.492	5/31
Education years (mean ± sd)	15.2 ± 3.4	15.04 ± 2.9	15.13 ± 4.63	16.6.± 1	15.5 ± 2	0.328	15.5 ± 3.0
Duration of disease (mean ± sd)	6.9 ± 9	6.6 ± 5.9	7.45 ± 7.8	4.45 ± 1.6	6.27 ± 4.6	0.855	–
University of Pennsylvania Smell Identification Test (UPSIT) (mean ± sd)	12.2 ± 4.0	22.4 ± 1.6	27.35 ± 1.2	30.75 ± 0.8	36.4 ± 1.2	**< 0.001**	32.8 ± 5.1
Hoehn & Yahr* stage (mean ± sd)	1.61 ± 0.5	1.26 ± 0.5	1.17 ± 0.3	1.25 ± 0.4	1.27 ± 0.4	0.142	–
UPDRS III* (mean ± sd)	24.3 ± 9.7	19.85 ± 8	20.3 ± 8.3	16 ± 0.8.3	19 ± 7/9	0.527	–
GDS score *(mean ± sd)	4.4 ± 1.2	4.1 ± 1.3	4.1 ± 1.5	4.6 ± 0.6	4.9 ± 1.0	0.484	4.5 ± 1.0
RBD score *(mean ± sd)	4.6 ± 2.5	4.3 ± 2.4	3 ± 2.2	3.58 ± 2.2	4.09 ± 1.9	0.372	3.1 ± 2.0
MOCA* score (mean ± sd)	27.8 ± 2.0	27.8 ± 1.7	27.4 ± 1.9	28.4 ± 1.5	27.4 ± 1.9	0.384	28.4 ± 1.1
HVLT Immediate Recall* (mean T-score)	0.17	−0.14	−0.87	−0.7	0.41	0.08	–
Benton Judgment Of Line Orientation (JoLO) (mean T-score)	−0.3	0.03	−0.73	−0.8	−0.17	0.696	–
Semantic Fluency (mean T-score)	0.21	−0.16	−0.45	−0.52	0.41	0.523	–
Symbol Digit Modalities Score (mean T-score)	−0.36	0.09	−0.48	−0.62	0.11	0.551	–

Bold values indicate significant differences

***p-value* of one-way ANOVA analysis for age at diagnosis, gender, handedness, education years and disease duration and one-way ANCOVA for UPSIT, H&Y stage, UPRDS part III, GDS scale, MoCA score, HVLT score, JoLO score, semantic fluency score, and symbol digit modality score, with age, sex, education years and disease duration as co-variates

*Age = age at diagnosis for PD patients and age of image acquisition for healthy controls in years, H & Y stage = Hoen and Yahr stage, UPDRS III = Unified Parkinson's Disease Rating Scale part III, GDS = Geriatric depression scale, RBD = REM sleep behavior disorder, MoCA = Montreal Cognitive Assessment, HVLT = Hopkins Verbal Learning Test dataset was acquired on a 3 T Siemens scanner, producing 64 Diffusion MRI (repetition time = 7748 min, echo time = 86 min; voxel size: $2.0 \times 2.0 \times 2.0$ mm^3; field of view = 224×224 mm) at b = 1000s/mm^2 and one b0 image along with a 3D T1-weighted structural scan (repetition time = 8.2 min, echo time = 3.7 min; flip angle = 8°, voxel size: $1.0 \times 1.0 \times 1.0$ mm^3; field of view = 240 mm, acquisition matrix = 240×240).

Diffusion MRI processing

The Diffusion MRI data were corrected for subject motion, eddy current distortions, and susceptibility artifacts due to the magnetic field inhomogeneity using Explore DTI toolbox (Leemans et al. 2009). We also preformed quality control analysis on the subject's signals based on the goodness-of-fit value given in QSDR reconstruction of fibers (Leemans et al. 2009). Each QSDR reconstruction file has a goodness-of-fit value quantified by R2. For example, an R82 indicates a goodness-of-fit between of the subject and template of 0.82 total. We excluded cases in which the R2 value did not reach a threshold of 0.6.

Between groups analysis

The diffusion data were reconstructed in the MNI space using q-space diffeomorphic reconstruction to obtain the spin distribution function (SDF) (Yeh and Tseng 2011), to detect the differences between groups of PD patients (anosmia and normal, mild, moderate, or severe loss of olfaction, and severe microsomia and normal, mild or moderate loss of olfaction). As healthy controls most had normal olfaction or mild micrsomia, we were unable to perform between group analyses for this group (see Participants Section).

Connectometry is a novel approach in the analysis of diffusion MRI signals that tracks the difference of white matter tracts between groups, or correlation of white matter fibers with a variable interest. Connectometry approach extracts the SDF in a given fiber orientation, as a measure of water density along that direction. A lot of diffusion indices are derived from spin density, that is, SDF, and quantitative anisotropy (QA) which is one of them (Yeh et al. 2013). QA of each fiber orientation gives the peak value of water density in that direction or tracts with significant correlation to a variable of interest.

In this first step of our analyses, diffusion MRI connectometry was used to identify white matter tracts in which QA was significantly different between two groups with various degrees of olfaction dysfunction (contrast or between-group analysis). Resulting uncorrected output was corrected for multiple comparisons by false discovery rate (FDR). A deterministic fiber tracking algorithm was conducted along the core pathway of the fiber bundle to connect the selected local connectomes (Yeh et al. 2013). Tracts with QA > 0.1, angle threshold lesser than 40° and tract length greater than 40 mm were included. To estimate the false discovery rate, a total of 2000 randomized permutations were applied to the group label to obtain the null distribution of the track length. Permutation testing allows estimating and correcting the false discovery rate (FDR) of Type-I error inflation due to multiple comparisons.

Connectometry

Diffusion MRI connectometry was used to study the effect of UPSIT. A multiple regression model was used to investigate correlation of UPSIT score with white matter QA, in 85 PD patients and 36 healthy controls, considering age, sex, GDS, MoCA, handedness as covariates of the model for both groups, and number of relatives with PD and H&Y stage as covariates for the PD group. The SDF was normalized. A T-score threshold of 2 was assigned to select local connectomes, and the local connectomes were tracked using a deterministic fiber tracking algorithm. A length threshold of 40 mm was used to select tracks. The seeding density was 50 seeds per mm^3. To estimate the false discovery rate, a total of 2000 randomized permutations were applied to the group label to obtain the null distribution of the track length. The analysis was conducted using publicly available software DSI Studio (http://dsi-studio.labsolver.org).

Results

PD patients in the five groups were matched in their age, sex, disease duration and education years (analysis of variance (ANOVA) or Kruskal–Wallis rank test significance > 0.05). Patients were also matched based on their degree of motor impairment in the five groups after controlling for age, sex and disease duration (analysis of covariance (ANCOVA) test significance > 0.05) (UPDRS-III p-value = 0.527, and H&Y scale p-value = 0.142) (Table 1). Patients were also matched in their GDS, MoCA score, HVLT, JoLO and other cognitive scores (Table 1).

Eleven patients in the anosmia group, eight patients with severe macrosomia, three patients with moderate microsomia, three patients with mild microsomia, and three patients with normal olfaction were at their stage 2 of H&Y, indicative of bilateral involvement without disturbance in balance. The rest of the patients were in stage 1 of H&Y scaling compatible with mild symptoms to unilateral involvement. None of the patients in any of the groups had MoCA score below 19, indicative of the absence of cognitive impairment in this group of PD patients.

Between-group analyses

Table 2 shows results of the between-group comparison of white matter QA for patients with anosmia and other groups. As expected, patients with anosmia and normal olfaction had the highest number of fibers with significant different QA (bottom line in Table 2), that is, left inferior longitudinal fasciculus, bilateral Fornix, bilateral MCP, bilateral cingulum, bilateral corticospinal tract (CST) and body, genu and splenium of corpus callosum (CC) (FDR = 0.0013) (Supplementary Fig. 1). Comparing fibers of patients with total loss of olfaction with those with mild to moderate and severe olfaction dysfunction yielded lower differences. These are depicted in regressing rows in Table 2 and supplementary Figs. 2, 3 and 4. Comparing results from contrast analysis of patients with severe macrosomia and normal olfaction yielded bilateral CST, MCP, cingulum, Fornix and left ILF (Table 3) (FDR = 0.0015). There were almost the same results when groups with the largest contrast in olfaction scores were compared, that is, between patients with anosmia and normal group, except for the CC. The results of the between-group analysis for patients with severe macrosomia is depicted in Supplementary Figs. 5, 6, and 7.

Multiple regression analysis

Results from multiple regression models in Diffusion MRI connectometry revealed areas where white matter QA was correlated with UPSIT score in each of the five groups of PD patients, but no significant results in healthy controls.

In line with the results from the between-group analysis, the UPSIT score could predict the highest number of fibers with significant positive/negative correlation in the QA, in the multiple regression models in anosmia and sever microsmia groups. These are shown as the two bottom rows of positive and negative correlation in Table 4. These fibers were similar to fibers that

Table 2 Results of between group analysis of fibers with decreased quantitative anisotropy in Parkinson patients with anosmia compared to those with severe, moderate, and mild microsmia and with patients with intact olfaction

Severe microsmia*						
Moderate microsmia*						
Mild microsmia*						
Normal Olfaction						
Body, genu and splenium of corpus callosum	Bilateral CST**	Bilateral Cingulum	Bilateral MCP**	Left fornix	Right fornix	Left ILF**

*FDR = false discovery rate values indicates the significance of difference in quantitative anisotropy of fibers in each comparison, anosmia with sever microsmia (*FDR = 0.0201*), anosmia with moderate microsmia (*FDR = 0.0468*), anosmia with mild microsmia (*FDR = 0.0212*), and anosmia with normal olfaction (*FDR = 0.0013*)

**CST = corticospinal tract, MCP = middle cerebellar peduncle, and ILF = inferior longitudinal fasciculus

Table 3 Results of between group analysis of fibers with decreased quantitative anisotropy in Parkinson patients with severe microsmia compared to those with moderate and mild loss of olfaction and those with normal olfaction

Moderate microsmia				
Mild microsmia				
Normal Olfaction				
Bilateral CST	Bilateral Cingulum	Bilateral MCP	Left ILF	Bilateral Fornix

*FDR = false discovery rate values indicates the significance of difference in quantitative anisotropy of fibers in each comparison, severe microsmia with moderate microsmia (*FDR = 0.0765*), severe microsmia with mild microsmia *(FDR = 0.0765)*, and severe microsmia with normal olfaction (*FDR = 0.0015)*

**CST = corticospinal tract, MCP = middle cerebellar peduncle, and ILF = inferior longitudinal fasciculus

Table 4 Results of multiple regression analysis of fibers in which quantitative anisotropy significantly predicted UPSIT score in each of PD patients in each of the five groups with age, sex, GDS, MoCA, handed, number of relatives with PD and H&Y stage considered as covariates in the model

<table>
<tr><td rowspan="6">Positive Correlation</td><td>0.0308</td><td colspan="2">Normal olfaction</td><td></td><td></td><td></td></tr>
<tr><td>0.0307</td><td colspan="2">Mild microsmia</td><td></td><td></td><td></td></tr>
<tr><td>0.0223</td><td colspan="4">Moderate microsmia</td><td></td></tr>
<tr><td>0.0929</td><td colspan="5">Severe microsmia</td></tr>
<tr><td>0.0585</td><td colspan="5">Anosmia</td></tr>
<tr><td>FDR*</td><td>Bilateral Cingulum</td><td>Bilateral MCP**</td><td>Bilateral CST**</td><td>Left UF**</td><td>Right IFOF**</td></tr>
<tr><td colspan="7"></td></tr>
<tr><td rowspan="6">Negative Correlation</td><td>FDR*</td><td colspan="3">Genu, Splenium and Body of Corpus Callosum</td><td>Bilateral Fornix</td><td>Left IFOF</td></tr>
<tr><td>0.0247</td><td colspan="5">Anosmia</td></tr>
<tr><td>0.0668</td><td colspan="4">Severe Microsmia</td><td></td></tr>
<tr><td>0.0195</td><td colspan="4">Moderate Microsmia</td><td></td></tr>
<tr><td>0.0832</td><td colspan="4">Mild Microsmia</td><td></td></tr>
<tr><td>0.0286</td><td colspan="4">Normal olfaction</td><td></td></tr>
</table>

*FDR = false discovery rate values indicates the robustness of correlation of the fibers in each group e.g. UPSIT score in PD patients with normal olfaction positively predicted quantitative anisotropy of bilateral cingulum and bilateral MCP with an FDR of 0.0308

**CST = corticospinal tract, MCP = middle cerebellar peduncle, UF = uncinate fasciculus, and IFOF = inferior fronto-occipital fasciculus

had previously shown the difference between anosmia and severe microsmia group and other groups. Interestingly the body, genu and splenium of CC and bilateral fornix had significant negative correlation, and bilateral cingulum and bilateral MCP had significant positive correlation with UPSIT scores in all groups (Table 4). There were also white matter areas that had the significant positive correlation in QA with UPSIT score of all groups but did not show any significant difference when comparing groups with various degrees of olfaction dysfunction (between group analysis). These were the left uncinate fasciculus in all groups and the right inferior fronto-occipital fasciculus (IFOF) in patients with severe to complete loss of olfaction (Table 4).

Connectivity in none of the white matter fibers from HCs could predict UPSIT score in the multiple regression analysis, in line with cohort specific results of UPSIT score in the regression model of PD patients.

Discussion

Current results

The present study investigated the ability of DMRI connectometry to identify neural underpinnings of different degrees of olfaction dysfunction in early PD. We aimed to identify signature white matter areas with increased/decreased connectivity in PD patients with complete or severe loss of olfaction compared to those with moderate, mild, or no olfaction identification deficit. This is the first study from the PPMI database that addresses this issue.

Herein we implemented Diffusion MRI Imaging to identify underlying brain structural changes between different subgroups of olfaction dysfunction and those with normal olfaction in drug-naive PD patients. First, we tried to answer the question whether the classification of olfaction function based on UPSIT score could reveal logical structural differences in white matter connectivity in early PD. Table 2 depicts how white matter structural differences increase with incremental changes in olfaction deficit, with most evident changes observed when comparing those with normal olfaction and those with the total loss of sense of smell.

When comparing local connectome patterns of PD patients with anosmia and patients with mild microsmia or normal olfaction, we identified decreased connectivity in bilateral CST, bilateral MCP and bilateral cingulum and corpus callosum. Comparing the anosmia and moderate microsmia group, only CC and bilateral CST were revealed. Finally, corpus callosum was the only fiber with decreased QA in anosmia, compared to severe microsmia. Similar fibers were observed in step-wise comparison of patients with severe microsmia with the other three group. Right and left fornix and left ILF were among fibers that only showed significance comparing those with intact olfaction or mild microsmia with severe microsmia and anosmia. Overall, limbic fibers such as fornix and cingulum and associational integrative fibers, such as ILF could better discriminate milder degrees of olfaction deficit in early PD. Table 4 also confirms this claim, as connectivity of bilateral fornix, cingulum, and IFOF being able to predict UPSIT score in different groups. Olfaction and limbic circuits are entangled in anatomy and function and new models in PD pathology suggest an even earlier involvement of the limbic areas in PD along with the brainstem and even before lewy body pathology spreads through the olfactory bulb (Luo et al. 2016). Also, a recent study implemented network statistics to identify decreased connectivity and reduced networking patterns in limbic circuits as well as olfactory and motor circuits in early PD (Nigro et al. 2016), compatible with the Braak staging and model of PD spread (Braak et al. 2003).

The cingulum is a complex white matter tract, interconnecting cingulate, prefrontal, and temporal cortical areas, including parahippocampal, septal and entorhinal gyri (Schmahmann et al. 2008). Anterior cingulum shows significant atrophy in early PD, in line with a possible contribution of this fiber in preclinical phase of the disease (Tessa et al. 2014). Previous research have also revealed increased FA in white matter areas adjacent to primary olfactory cortices, including entorhinal cortex, in PD patients with anosmia compared to controls, which are possibly conveyed through cingulum bundle. Moreover, parahippocampal and septal areas are implicated in higher order olfaction interpretation, further supporting a role for cingulum in PD related mild olfactory deficit.

The fornix is a subcortical white matter tract of the limbic system involved in PD depression, loss of episodic memory, and poor visuospatial processing (Hall et al. 2016), and is a prodromal marker of depression in early PD (Ansari et al. 2016). It is possible that difference in connectivity of fornix between patients with normal olfaction or mild microsmia and those with severe/complete olfaction loss, rise from severity of other non-motor symptoms in the latter group, although the difference might not be as high as to reach significance (Table 1).

Next, connectivity of bilateral CST could differentiate between those with anosmia or severe microsmia and PD patients with UPSIT scores compatible with normal olfaction to moderate microsmia. Bilateral middle cerebellar peduncles not only could predict UPSIT score in all groups, but also showed different microstructure in between group contrast analyses. Recently, we identified bilateral CST and bilateral MCPs to be associated with RBD in early PD (Ansari et al. 2016), suggesting shared neural pathological substrates for RBD and olfaction. We also identified right CST, cingulum and bilateral fornix to be associated with comorbid depression in early PD patients with RBD *(unpublished, Ghazi Sherbaf* et al.*)*. However, PD patients in our five groups had comparable RBD and GDS scores (GLM *p-value* = 0.484, and 0.372 respectively), and therefore these results cannot be explained by concomitant depression or RBD in patients with more severe smell identification deficit.

Persistent results from both within and between groups were observed in bilateral CST, bilateral MCP, bilateral cingulum and in corpus callosum. MCP is an area with significant decline in diffusivity in early PD (Zhang et al. 2016), and lower olfaction identification threshold predicts faster motor progression (Rahmani and Aarabi 2017) in PD patients, in line with involvement of CST and other pyramidal tracts in early PD.

In a complementary analysis we showed that QA of several white matter areas could be predicted through a multiple regression model with UPSIT score, age, sex, depression, RBD, cognitive scores, etc. Connectivity of none of the white matter fibers from MNI atlas showed correlation with UPSIT score using the same model in healthy controls. This fact puts further spin on validity of UPSIT score in identification of prodromal olfaction deficit, in various degrees, in PD. UPSIT is a globally used test to identify olfaction identification dysfunction in PD patients (Rodriguez-Violante et al. 2014). Our results confirm a discriminative role for UPSIT in identifying PD specific changes in white matter microstructure.

Integrating results from the current study with studies addressing NMS for identification of early PD (Ansari et al. 2016; Rahmani and Aarabi 2017), provides compelling evidence that the clinical phenotype of prodromal PD can be well represented through imaging markers, such as diffusion parameters of white matter areas identified as signature markers for prodromal PD. Interestingly we observed both positive and negative associations between connectivity and UPSIT score in different fibers. Reports exist that some white matter areas show a paradoxical increase in FA in baseline PD patients, and interestingly show a steeper decline in FA (along with increase in RD and AD). Mole and his colleagues revealed an increase in FA of motor pathways including in bilateral corticospinal tract in early PD, which they attributed to selective neurodegenerative pathology or a compensation response in early PD (Mole et al. 2016). Dr Zhang et al. reported similar results in their longitudinal analysis of the PPMI database. FA value of the contralateral IFOF and body and splenium of corpus callosum was higher in drug naïve PD patients compared to healthy controls (Zhang et al. 2016).

Of interest also, Ibarretxe-Bilbao et al. investigated for the first time whether USPIT scores correlated with white matter degeneration in areas adjacent to rectus gyrus and primary olfactory areas through a voxel-based analysis (Ibarretxe-Bilbao et al. 2010). FA values in these areas were higher in groups of PD patients with better olfaction function and correlated with UPSIT scores in all PD patients (Ibarretxe-Bilbao et al. 2010). It is possible that observed directionality in our results from multiple regression analysis is due to any/all of the above possibilities. It is plausible to deduce that UPSIT scores are predicted better by areas that are already damaged or in high potential of more rapid degeneration, i.e. compensatory increase in diffusometric.

Olfaction dysfunction as a clinical marker

Smell insufficiency is a precursor to neurodegeneration (Postuma and Berg 2016) and robust evidence supports a predictive value of olfactory dysfunction for PD. When compared to NMS-like REM sleep behavior disorder (RBD), the relative risk for development of PD in patients with microsomia is relatively low. However, simplicity and sensitivity of smell identification measurement has made microsomia an interesting target for detection of PD. Increased risk for development of PD conferred by microsomia is identified to be about 5 times the risk conferred by subtle motor dysfunctions (Munhoz et al. 2015).

As mentioned, cross-cultural as well as intermethodological differences in identification of smell insufficiency, high prevalence of mild to moderate microsomia in general population, as well as other neurodegenerative conditions, other than PD, that are associated with macrosomia, provide reasons against use of olfaction identification in as a prodromal marker of PD.

Combination of clinical, structural, and/or serum biomarkers to achieve more robust prediction of prodromal PD has gained increasing momentum in the past few years (Ross et al. 2008). Studies successfully combined olfaction dysfunction with RBD to give a risk of 65% for conversion to PD, over a 5-year period (Visanji and Marras 2015). In another study olfaction dysfunction in a group of patients with iRBD was able to predict PD with a pre-motor interval of more than ten years, enough to initiate neuroprotective interventions (Munhoz et al. 2015). Olfactory deficit in PD strongly correlates with faster motor progression, and with more sever RBD in early PD. PD patients with co-morbid RBD and olfactory dysfunction are more likely to exhibit cognitive dysfunction and a more rapidly progressive motor decline. In a successful attempt to combine imaging markers with clinical markers, olfactory deficit combined with DAT deficit on imaging showed to be highly predictive of PD, with olfaction deficit being able to predict DAT decline in both prodromal and early PD stage (Jennings et al. 2017). Our results however, cannot provide evidence in support or reject of the combination of olfaction identification and other non-motor scores including RBD or GDS. Nonetheless, we provide evidence that olfactory dysfunction in early PD is supported by concomitant structural alterations in specific white matter areas.

Use of DMRI connectometry

It is worth mentioning advantages and disadvantages of connectometry approach and the specific parameters of this method, over conventional DMRI diffusion metrics. Previous studies have revealed microstructural differences between PD patients with olfactory impairment and healthy controls, indexed by reduced fractional anisotropy (FA) values in white matter adjacent to gyrus rectus and primary olfactory cortex (Ibarretxe-Bilbao et al. 2010). In a DWI hypothesis-free statistical parametric mapping Scherfler et al.2006 found significantly increased diffusivity along olfactory tracts in patients with clinical microsomia compatible with reduced integrity and degeneration of white matter in this area (Scherfler et al. 2006). A voxel-based analysis of DTI later revealed areas with decreased FA and increased mean diffusivity (MD) in the orbitofrontal cortex which together with the rectus gyrus comprise the secondary olfactory cortical areas.

The main diffusivity parameter used by DMRI connectometry, is quantitative anisotropy which is derived from spin distribution function. Unlike conventional diffusion parameters such as FA and MD, QA is based on water density of a given voxel but not diffusivity. FA is indeed a common diffusion parameter, reflecting neuropathologic conditions such as axonal loss and demyelination, and MD is a more sensitive scalar measure of total diffusivity regardless of orientation (Wiltshire et al. 2010). In contrast, QA is defined for any given direction within a voxel and therefore, gives a better spatial resolution identifying microstructural disturbances in areas with kissing or crossing fibers, compared to FA (Yeh et al. 2015).

Conclusion

The quest for searching for biomarkers of Parkinson initiation and progression has made significant forward steps through identification of microstructural and subtle neural changes in early PD. Understanding the neurological substrates of olfaction dysfunction in early PD, would therefore, be eye-opening for the combination of clinical markers in future studies. Any deduction from the current study is limited by the small number of PD patients in each of the groups. Last but not the least, depression scores, smell identification tests and other batteries used by the PPMI database, results from patients' cognitive state and other tests performed. These tasks frequently require one or more of the cognitive skills to be intact for successful completion (Murphy et al. 1991), which might question validity and reliability of their results.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest, nor have they received any funding from any organization or granting bodies which might pose any conflict of interest.

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