Serial 3T MRI in early Parkinson's to validate PD progression markers: Rationale and MRI analysis plan for nigral depigmentation at baseline, 6- and 12-months' follow-up.

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Background and rationale:

InsIghtPD is a single centre (University of Nottingham) case-control observational longitudinal study to prospectively evaluate nigral depigmentation and explore other MRI metrics as potential progression markers in early Parkinson's disease (Trial registration: https://classic.clinicaltrials.gov/ct2/show/NCT05631158). The study procedures plan for up to 2 years follow-up with six-twelve monthly visits including 3T MRI (neuromelanin (NM)-brainstem and multimodal brain MRI) combined with a battery of clinical assessment of Parkinson's symptoms, mood, cognitive, life quality and sleep. In this analysis protocol, we summarise the rationale and hypotheses, primary and secondary outcomes, followed by the documentation of data processing methods and the statistical analysis plan for a selection of MRI metrics from available data using baseline, 6 months and 12 months follow-up.

Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, is characterized by a long preclinical phase followed by progressive motor and non-motor symptoms. There is substantial heterogeneity in the trajectories of disease progression, which currently cannot be individually predicted. The emergence of promising disease-modifying treatment approaches highlights the need for better prediction and monitoring of disease progression to inform drug efficacy and guide personalised treatment [1], [2].

Dopamine imaging effectively quantifies striatal dopaminergic deficits but faces challenges in tracking disease progression. Neuromelanin-sensitive MRI (NM-MRI) is a promising novel approach sensitive to the neuromelanin-iron complex in the substantia nigra [3], [4]. While its diagnostic value in PD has been established in case-control studies [5]–[15], its potential as a progression biomarker remains underexplored [16], [17] especially in the early disease stage when disease modifying treatment is expected to be most effective. Hence, we focus our progression marker assessment on the substantia nigra to best reflect changes in early clinical PD (Braak&Braak stage 3) and primarily on depigmentation. Secondary nigral outcome metrics are chosen based on the extant literature (such as free water, kurtosis and susceptibility) and our discovery work. Additional brain metrics with promising potential as progression marker will be studied using appropriate explorative design (such as deformation (DBM) and brain age gap) [17] and will be described elsewhere. This is to address a knowledge gap of minimal detectable change of these MRI metrics in the early clinical phase of PD, whether early change is linked to clinical subtypes of slow and fast progressors. Also, little is known how well these change metrics separate PD progression from effects of physiological ageing.

Aim and objectives:

The common aim across these MRI metrics is to assess their power as progression marker specifically to detect disease progression earlier than clinical deterioration, and thereby to predict future clinical progression which will be particularly helpful in the context of future disease-modifying trials. In addition, little is known about their values in disentangling the PD-related pathological changes and the physiological ageing effect within a shorter timeframe compared to the existing literature, such as 1 or 2 years.

Therefore, this study was designed to describe the trajectory of several MRI metrics in recently diagnosed Parkinson's pathology and in healthy controls over 2 years with concurrent clinical follow-up for validation. Here we describe the primary and secondary hypotheses and the analysis plan for the main nigral MRI metrics.

Hypotheses:

Primary:

1. Dorsolateral nigral depigmentation (assessed by predefined serial NM-MRI metrics) progresses over 6 and 12 months in early PD, but not in healthy controls.

2. Dorsolateral nigral depigmentation rate at 6 months predicts depigmentation at 12 months.

3. Dorsolateral nigral depigmentation rate at 6 and 12 months predicts future clinical deterioration*

Secondary hypotheses:

1. Using voxel-based and novel NM-MRI metrics, nigral depigmentation in early PD a) can be reliably detected at 6 months and 12 months; b) can be separated from physiological ageing effects at baseline, 6 and 12 follow-up months.

2. Free water, kurtosis, and iron content progress (dorsolateral nigral regional and voxel-based) in early PD over 6 and 12 months, compared to the baseline.

3. Nigral depigmentation rate (dorsolateral and voxel-based) is associated with increase of iron content (QSM-MRI) and increased free water at 6 and 12 months.

4. Estimated brain age accelerates in early PD over 6 and 12 months.

*Will be detailed in a separate analysis plan defining PD subtyping and related subgroup and clinical symptom association as further secondary analyses.
Additional analyses will be considered exploratory.

Required data:

- For addressing the depigmentation related hypotheses, NM-MRI and structural MRI (registration use) at BL, 1st FU and/or 12 months FU.

- For addressing the non-NM based hypotheses, we also need regional and voxel-based metrics derived from diffusion and SWI MRI at the respective timepoints. The high-resolution T1 structural MRI will be used for brain age estimation.
- Demographics including age, sex, and the exact duration between each visit of all patients and healthy controls in months.

Outcome metrics:

- ### Primary regional nigral depigmentation metrics

    - Dorsolateral nigral pigmentation index (dlNNMC) measures at each timepoint:

dlNNMC=(μ_SN - μ_BG)/μ_BG, where μ_SN corresponds to the mean intensity value over a region of interest (ROI) in the SN and μ_BG corresponds to the mean intensity value over a delineated background area within the cerebral peduncles.

    - Trajectory description:

To better understand the depigmentation trajectory over time, we will plot dINNMC measures at the BL, 6months and 12-month time points in both PD and HC.

    - The loss of dINNMC (DINNMC_loss) will be measured as annual difference of dINNMC:

DlNNMC_loss= 12 * (dlNNMC_BL - dlNNMC_FU)/Nmonth, where Nmonth is the number of months between BL and FUs.

- ### Secondary metrics:

    - Voxel-based NM-MRI metrics:

A voxel-based analysis was performed using the two FUs vs. BL NM data. For each voxel, a binarized classification was performed and the area under the curve (AUC), ranging from 0-1, was calculated as it is easier for quantification and comparison. Instead of using correction for multiple comparisons, we reported all the AUC values voxel-wise to demonstrate the topology of classification performance in the brainstem region (Figure 2 left).

    - Novel NM-MRI metrics:

1). Left/right radius: Corresponds to the euclidean distance between the centre of mass of the posterior-weighted left/right intensities in the SN label and the MNI152 space point in the cerebral aqueduct defined as (X = 90mm, Y = 92mm, Z = 58mm).

2). Angle: Corresponds to the angle subtended by the vectors defining the left and right radii.

    - Other MRI metrics:

1). Regional and voxel-based free-water and Kurtosis based on diffusion imaging:

Free-water and kurtosis maps will be generated using the analysing pipelines [24]. The regional mean values of free-water and kurtosis at each time point will be extracted from the dorsalateral nigral region defined below. The voxel-based longitudinal change map will be computed using the 6 months Vs. BL, and 12 months vs. BL with the same method as the voxel-based analysis for NM.

2). Regional and voxel-based QSM based on SWI:

The QSM will be generated using the in-house GUI-based toolbox for all the time points. The regional mean values of QSM at each time points will be extracted from the dorsalateral nigral region defined below. The voxel-based longitudinal change map will be computed using the 6 months Vs. BL, and 12 months vs. BL with the same voxel-based analysis.

3). Brain age estimation:

This metric has proven to be associated with disease status and progression [19]. Based on 5000 HC T1WI structural training datasets from UK-Biobank, we will calculate the accelerated brain age in our PD cohorts at the 6- and 12-months' time points, using https://github.com/SPMIC-UoN/deltab.

MRI acquisition:

The NM-MRI was acquired on a 3T GE Discovery MR750 scanner was based on test-retest and visual appearances of tissues of interest. The reproducibility data was obtained by testing on four control subjects. The final NM-MRI sequence was optimised to be: Fast Spin Eecho higher resolution_SB_2.0mm, flip angle 150, TE min full, TR 900, Echoes 1, ETL 3, Multi-phase 3, FOV 18.0, slice thickness 2.0, spacing 0.2, number of slices 12, saturation bands (fat) 2 (superior/inferior). This sequence selection was found to be excellent and superior to alternative sequences from the literature.
For free-water and QSM assessment, we also included an advanced diffusion for NODDI and SWI protocol. High-resolution T1WI structural image was acquired as well as detailed elsewhere.

NM-MRI image analysis:

- Initial Quality Control (QC):

The NM-MRI sequence used in the study includes three volumes, which can be averaged to improve signal to noise. A quality control procedure will be carried out to determine which of the three volumes are usable for averaging and which ones have to be discarded (e.g., due to severe motion or other artefacts in the dorsolateral nigral region). Firstly, the three repeats will be rigidly registered to a coarse common space defined by their average in iterative fashion. Then, measures based on pairwise regression of intensities will be computed to flag up potentially problematic volumes. If any volume is flagged up, all three volumes will be visually reassessed by two independent raters with >6 years of experience of assessing image quality and a consensus will be reached in case of any discrepancies. This procedure is summarised in Figure 1

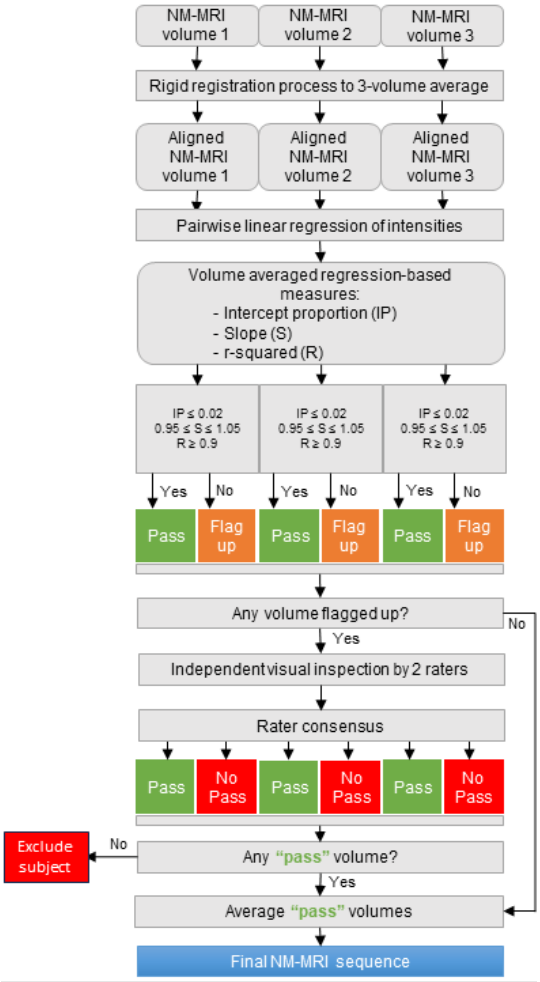

Figure 1. The initial QC pipeline illustrated as flowchart

- Data Processing:

1. Structural T1WI MRI processing: Structural T1WI images are first bias field corrected using N4ITK [18], then the brain parenchymal area is extracted with ROBEX [19] and a final bias field correction is run on the brain extracted image. Finally, a non-rigid registration of the processed image and the brain extracted MNI152 T1WI template is performed using MIRTK [20]. The resulting deformation field is subsequently used for the processing of the neuromelanin-sensitive image described below.
2. NM-MRI processing: A processing pipeline was developed in order to bring the neuromelanin-sensitive images of each participant into a common space following a procedure similar to that of Pszczolkowski et al [23]. Different from the earlier version is that here on each iteration of the template construction procedure, the individual native to MNI transformations are used to create a prior map for each label, which are then propagated back to each individual native space to compute individual posterior maps for each label. The posterior maps for SN are then used as weights to create the individual synthetic images that are subsequently averaged.
3. Post-processing QC of NM-MRI: Due to the restricted brainstem coverage of NM-MRI, a further quality control procedure will be carried out on the NM-MRI sequence after alignment into MNI152 space. To this end, (see Figure 2) the spatial overlap between a brainstem ROI mask and brainstem voxels in the NM-MRI image with intensities greater

than a threshold will be computed. This threshold corresponds to 0.5 times the mode of nonzero brainstem intensities (computed as the peak of the intensity histogram). If this coverage estimation is less than 90%, the dataset will be excluded from further analysis.

- Outcome of QC:

The flagged-up reasons and numbers, the rescuable cases, and the final attrition rate of BL, 6 month and 12 months will be calculated based on the QCs.

- Included time points:

For each pair-wise comparison only time point pairs with complete data will be included but may be retained for trajectory analysis if single time points are missing.

ROI definition of dorsolateral substantia nigra

We will establish the ROIs of the dorsolateral substantia nigra using anatomical a priori knowledge and a region-growing method. Starting from the two MNI152 coordinates representing the centroids of the mass for the bilateral nigrosome-1 (X = 10.9 mm, Y = -21.6 mm, Z = -14.6 mm) and (X = -10.1 mm, Y = -22.0 mm, Z = -14.9 mm) [21], we will grow these seeds into a sub-nigral areas (referred as "hot spot") containing the voxels that shows the most depigmentation around the time of diagnosis in PD. This is based on the rationale that the depigmentation is not homogenous across substructures of the substantia nigra or disease stage, and the sub-nigral areas showing most depigmentation at the time of diagnosis in PD can best discriminate PD from non-neurodegenerative or controls, will undergo the most depigmentation over the first years from diagnosis. To achieve that, a voxel-wise AUC map will be first obtained from an independent cross-sectional (N3iPD) dataset, including clinically uncertain parkinsonism. It will then be made symmetrical by averaging the AUC values of the two hemispheres. The seed-growing will be initiated on the slice of the centroids and restrained to voxels with a symmetrical AUC value greater than 0.7. One iteration of morphological closing will be applied to the resulting ROIs to obtain the final 'hot spot'. We will use a 6-connectivity kernel in all operations. Finally, this hotspot will also be compared with the AUC maps obtained from the cross-sectional data of the InsightPD and PaMIR [22] for sense checking.

Statistical method for testing primary hypothesis with currently available data:

(PH1): To measure whether the dINNMC progresses over 6 and 12 months in early PD, the annual change of measures at 6 months and 12 months will be compared with zero, separately using one-tailed single group t-test. The same tests will be used for testing whether the dINNMC in HC cohort changes over time. To compare the changes between HC and PD, we will perform an unpaired t-test at each time point.

(PH2): To test whether dINNMC rate at 6 months predicts depigmentation at 12 months, a linear regression analysis will be performed by using 6 months rate as the dependent variable and 12 months as the independent variable.

(SH1): Voxel-based analysis based on FSL version 6.0.5 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise/UserGuide) will be conducted to address whether the nigral depigmentation in early PD can be reliably detected at 6 months and 12 months, with contrasts of the follow-up time points vs baseline in HC and PD, respectively. Similar approach will be

used to address whether the nigral depigmentation in early PD can be separated from physiological ageing effects at baseline, 6 and 12 follow-up months with a contrast between the changes of PD vs the changes of HC at each time point.

For all the statistical analyses, software tools such as FSL and Matlab will be used. P-value <0.05 will be considered as the threshold for statistically significant differences in t-test analyses and correlation analyses.

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