# **Supplementary Material**

# **Methods**

*Imaging*

All 17 iRBD subjects underwent [18F]FDG-PET brain imaging twice 3.6 ± 0.6 years apart (baseline 2014-2015; follow-up 2018) and [123I]FP-CIT-SPECT 3.9 ± 3.3 (range 0.4 – 13.5) months before baseline [18F]FDG-PET.15 [123I]MIBGs (mean 185 MBq) were performed 3.9 ± 2.3 (range 0.6 – 8.6) months before baseline [18F]FDG-PET, another two 10.1 and 15.9 months after the second [18F]FDG-PET. The [123I]MIBG scans were re-analyzed at the UMCG: heart-to-mediastinum ratio (HMR) of [123I]MIBG-binding was calculated on 4-hour planar images using the Symbia.net™ Clinical Workflow System (Siemens Healthcare, Erlangen, Germany). A cut-off value of 1.45 was determined post-hoc to give 100% sensitivity and specificity in differentiating normal from pathological results. In general, iRBD subjects with diseases (such as heart or kidney failure, myocardial infarction in the last five years, diabetes, amyloid or other neuropathy, pheochromocytoma) and/or intake of certain medications (such as tricyclic antidepressants, reserpine, opioids, labetalol, phenylpropanolamine, phenylephrine) which may affect [123I]MIBG results were excluded. Besides these exclusion criteria two iRBD subjects, i.e. subjects 9 and 15, had a history of a small myocardial infarction >10 years prior to [123I]MIBG. Both of them already had a pathological [123I]FP-CIT-SPECT at baseline, and one of these iRBD subjects already phenoconverted to PD. As Kane et al. described no effect of a history of myocardial infarction on heart-to-mediastinum ratio in [123I]MIBG1, we decided not to exclude them from the analysis. Researchers at both institutions were blinded throughout the study to the status of the [123I]MIBG results.

*Statistical Analysis*

Variables were tested for normality of distribution with the Shapiro-Wilk test. The following variables were considered to be non-parametric: [123I]MIBG-HMR values, UPDRS-III score, MoCA score, and years duration of iRBD. The following variables were considered to be parametric: PDRP z-scores, absolute PDRP z-score change between baseline and follow-up, PDRP z-score change-per-year, DAT-binding ratios, odor identification score, and age.

A Pearson correlation coefficient was used to correlate normally distributed variables. Correlations between the non-parametric variables as well as PDRP z-scores and DAT-binding ratios were compared with a two-sided Spearman’s rank correlation coefficient. All analyses were performed using SPSS v27 (SPSS Inc., Chicago, IL).

Two subgroups were formed: subjects with normal [123I]MIBG and subjects with pathological [123I]MIBG. The PDRP z-score change-per-year and absolute follow-up PDRP z-scores were predefined as the primary endpoints; the DAT-binding ratios and odor identification scores were predefined as the secondary endpoints. Due to small subgroup size, non-parametric tests were used: the Mann-Whitney-U-test to examine changes between both subgroups; a one-sample Wilcoxon signed-rank test for changes within subgroups.

# **Results**

*Overview*

Clinical, demographic, and imaging data of all subjects are summarized in Supplementary Table 1. All subjects showed in routine diagnostic brain MRI no typical findings for MSA and a normal brain MRI finding was described except for three subjects with white matter lesions. Upon visual reading of the [18F]FDG-PET scans, there were no visual signs of MSA (i.e., uptake in striatum and cerebellum appeared visually normal).

**Supplementary Table 1: Imaging and clinical data.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **MIBG at baseline** | **DAT-binding**  **status at baseline** | **No.** | **Gender** | **Age (years) at baseline** | **Age (years) at FU** | **Duration iRBD (years) at FU**  **[baseline to phenoconversion (years)]** | **PDRP z-score FU (change from baseline)** | **PDRP change-per-year** | **[123I]MIBG HMR value** | **Lowest DAT-binding ratio** | | **Odor identification score** | | **UPDRS-III at FU (change from baseline)** | **MoCA at FU (change from baseline)** |
| **putaminal** | **caudatal** | **baseline** | **follow-up** |
| normal | normal | 1 | M | 57.5 | 61.2 | 6.2 | -0.72 (+1.47) | 0.40 | 1.60 | 2.53 | 2.86 | *9* | 13 | 3 (-3) | 29 (+2) |
| 2 | M | 57.4 | 61.4 | 9.0 | 0.70 (+1.18) | 0.30 | 1.54 | 2.20 | 2.48 | 13 | 12 | 1 (-3) | 28 (-2) |
| 3 | M | 54.0 | 58.0 | 10.0 | 0.93 (+1.01) | 0.25 | 1.57**a** | 2.49 | 3.27 | 11 | 13 | 7 (+3)**b** | 27 (+1) |
| 4 | M | 67.1 | 70.1 | 28.0 | 1.77 (+0.35) | 0.12 | 1.72 | 2.17 | 2.88 | *6* | *8* | 0 (0) | 28 (0) |
| 5 | F | 68.3 | 72.5 | 10.3 | *2.07 (+2.*92)c | 0.68 | 1.74 | 2.51 | 2.87 | 14 | 14.0 | 2 (0) | 30 (+7) |
| abnormal | 6 | M | 57.8 | 60.4 | 7.6 | -0.77 (+0.80) | 0.31 | 1.23 | 2.53 | 2.82 | 12 | *10* | 0 (-1) | 27 (-1) |
| 7 | M | 64.5 | 67.5 | 5.0 | *2.60 (+3.30)*c | 1.10 | 1.23 | 2.27 | 2.74 | *8* | *6* | 4 (+2) | 29 (+3) |
| 8 | F | 70.1 | 74.7 | 7.6 | *4.41 (+2.23)* | 0.48 | 1.22 | 2.04 | 2.66 | *5* | *3.0* | 3 (-1) | 30 (+2) |
| abnormal | 9 | M | 66.9 | 69.9 | 6.0 | *2.44 (+1.44)*c | 0.48 | 1.10d | *1.61* | *2.15* | *10* | 12 | 4 (+2) | 27 (0) |
| 10 | M | 66.4 | 69.9 | 9.4 | *2.74 (+1.52)*c | 0.44 | 1.11 | *1.01* | *1.43* | *7* | *5* | 4 (+1) | 26 (-1) |
| 11 | M | 61.5 | 64.1 | 6.6 | *3.02 (+2.67)*c | 1.04 | 1.22 | *0.95* | *1.15* | *6* | *5* | 0 (0) | 30 (+3) |
| 12 | M | 65.4 | 69.1 | 9.7 | *3.72 (+0.97)* | 0.26 | 1.08 | *1.70* | 2.42 | *5* | *2* | 2 (-4) | 28 (+1) |
| 13 | M | 64.0 | 68.1 | 18.0 | *4.16 (+3.56)*c | 0.88 | 1.18 | *1.73* | 2.29 | 12 | 12 | 3 (-1) | 27 (-1) |
| 14 | M | 66.6 | 70.6 | 6.0 | *6.19 (+1.87)* | 0.48 | 1.04**a** | *1.82* | 2.26 | *0* | *0* | 3 (-2) | 29 (+7) |
| **15** | **M** | 65.9 | **69**.**9** | **16.0 [4.2]** | ***6****.****30 (+3****.****66)*** | **0**.**94** | **1**.**15d** | ***1****.****16*** | ***1****.****73*** | ***8*** | ***6*** | ***9 (+7)*** | **28 (+2)** |
| **16** | **M** | 63.2 | **67**.**2** | **8.0 [4.4]** | ***7****.****67 (+2****.****99)*** | **0**.**74** | **1**.**13** | ***1****.****18*** | ***2****.****08*** | ***0*** | ***0*** | ***7 (+6)*** | **28 (0)** |
| **17** | **M** | 49.9 | **53**.**9** | **8.0 [3.8]** | ***9****.****11 (+4****.****37)*** | **1**.**08** | **1**.**19** | ***1****.****96*** | **2**.**52** | ***2*** | ***2*** | ***15 (+14)*** | ***25 (+1)*** |
| MIBG/DAT normal. No. 1-5  median (range) | | | n = 5 | 57.5  (54.0 – 68.3) | 61.4  (58.0 – 72.5) | 10.0  (6.2 – 28.0) | 0.93 (-0.72 – 2.07)  (1.18 (0.35 – 2.92)) | 0.30  (0.12 – 0.68) | 1.6  (1.5 – 1.7) | 2.5  (2.2 – 2.5) | 2.9  (2.5 – 3.3) | 11  (6 – 14) | 13  (8 – 14) | 2 (0 – 7)  (0 (-3 – 3)) | 28 (27 – 30)  (1 (-2 – 7)) |
| MIBG abnormal, no phenoconversion. No. 6-14  median (range) | | | n = 9 | 65.4  (57.8 – 70.1) | 69.1  (60.4 – 74.7) | 7.6  (5 – 18.0) | 3.02 (-0.77 – 6.19)  (1.87 (0.80 – 3.56)) | 0.48  (0.26 – 1.10) | 1.2  (1.0 – 1.2) | 1.7  (1.0 – 2.5) | 2.3  (1.2 – 2.8) | 7  (0- 12) | 5  (0 – 12) | 3 (0 – 4)  (-1 (-4 – 2)) | 28 (26 – 30)  (1 (-1 – 7)) |
| Phenoconverters. No. 15-17  median (range) | | | n = 3 | 63.2  (49.9 – 65.9) | 67.2  (53.9 – 69.9) | 8.0  (2.6 – 4.7) | 7.67 (6.30 – 9.11)  (3.66 (2.99 – 4.37)) | 0.94  (0.74 – 1.08) | 1.2  (1.1 – 1.2) | 1.2  (1.2 – 2.0) | 2.1  (1.7 – 2.5) | 2  (0 – 8) | 2  (0 – 6) | 9 (7 – 15)  (7 (6 – 14)) | 28 (25 – 28)  (1 (0 – 2)) |

a[123I]MIBG after FU. b Artificially-elevated UPDRS-III due to back problems. **c**Developed suprathreshold PDRP z-scores at follow-up. dSubject 9 and 15 had a history of myocardial infarction >10 years before [123I]MIBG. Italics denotes pathological results, bold the phenoconverted subjects. FU=follow-up [18F]FDG-PET

*[123I]MIBG after Follow-up [18F]FDG-PET*

One subject (subject 14) of the 12 iRBD with pathological [123I]MIBG underwent [123I]MIBG after follow-up [18F]FDG-PET. This person had a pathological [123I]FP-CIT-SPECT at baseline. According to the literature there is no report of [123I]FP-CIT-SPECT being pathological prior to cardiac [123I]MIBG in any iRBD subject. So we presume that this subject would have had an abnormal [123I]MIBG at baseline.

One subject (subject 3) of the five subjects with normal [123I]MIBG underwent [123I]MIBG after follow-up [18F]FDG-PET. We presume that this subject would have had a normal [123I]MIBG at baseline as well, as it is unlikely that a pathological baseline [123I]MIBGwould (spontaneously) revert back to normal in a progressive neurodegenerative disorder; one study reported a decline of [123I]MIBG-HMR in PD over the course of about 3 years2.

*UPDRS-III and Olfactory Function in the Two Subgroups*

Of the 12 subjects with abnormal [123I]MIBG, 50% showed increased UPDRS-III at follow-up while in subjects with normal [123I]MIBG only one subject increased in UPDRS-III due to back problems.

At follow-up, only 1/5 iRBD subjects with normal [123I]MIBG presented with olfactory dysfunction whereas 9/12 iRBD subjects with pathological [123I]MIBG had hyposmia/anosmia. None of the subjects with normal [123I]MIBG worsened in odor identification score whereas 7/12 (58%) subjects with pathological [123I]MIBG showed decreased odor identification score at follow-up (see Supplementary Figure 2). Additional three subjects (25%) already had odor identification score of 0.0 or 2.0 at baseline.

*UPDRS-III and Olfactory Function in the Three Phenoconverted Subjects*

As previously described3, the phenoconversion to PD was diagnosed by the neurologist according to the UK Parkinson’s Disease Society Brain Bank diagnostic criteria and the diagnosis of PD had to be confirmed after three months. The three phenoconverters worsened in UPDRS-III from baseline to follow-up (see Supplementary Material Table 1). Two of them did not change in odor identification score that already was 0.0 and 2.0 respectively at baseline whereas subject 15 worsened from 8 to 6 points in odor identification score (see Supplementary Material Table 1). As recently published3, the UPDRS-III change-per-year correlated with the PDRP expression z-score change-per-year in the 20 iRBD subjects. This effect was maintained in the analysis of the 17 iRBD subjects in the current study: the UPDRS-III change-per-year correlated with the PDRP expression z-score change-per-year ( 0.495, *P* = 0.040).

*Correlation Analysis*

A negative correlation was found between [123I]MIBG-HMR values and PDRP z-scores at baseline and follow-up in all 17 subjects (baseline: ρ = -0.679, *P* = 0.003; follow-up: ρ = -0.650, *P* = 0.005, Supplementary Figure 1A-B).

[123I]MIBG-HMR values correlated significantly with baseline and follow-up odor identification scores (baseline: ρ = 0.542, *P* = 0.025; follow-up: ρ = 0.679, *P* = 0.003). Additionally, odor identification scores correlated inversely with PDRP z-scores at baseline and follow-up (baseline: *r* = -0.838, *P* < 0.0001; follow-up: *r* = -0.745, *P* = 0.001; see Supplementary Figure 1C-D).

[123I]MIBG-HMR values correlated significantly with putaminal (*P* = 0.001, ρ = 0.735) and caudatal DAT-binding (*P* = 0.00033, ρ = 0.767). Only lowest putaminal DAT-binding ratios correlated with both baseline and follow-up PDRP z-scores (baseline: *r* = -0.533, *P* = 0.028; follow-up: *r* = -0.538, *P* = 0.026).



**Supplementary Figure 1:** Correlation between [123I]MIBG-HMR (abnormal <1.45) and PDRP z-score (cut off >1.98) at baseline (1A) and follow-up (1B) and between odor identification score (abnormal ≤10) and PDRP z-score at baseline (1C) and follow-up (1D). Phenoconverters are marked ( ).

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**Supplementary Figure 2:** Odor identification score at baseline and follow-up according to [123I]MIBG status.

*Sequence of Imaging Changes*

It is an issue of discussion, in which sequence the employed three imaging biomarkers ([123I]MIBG scintigraphy, [18F]FDG-PET or [123I]FP-CIT-SPECT) show a reduction in signal, i.e. a pathologocial state. According to our data in two subjects, cardiac noradrenergic denervation has preceded both nigrostriatal dopaminergic degeneration as well as altered cerebral glucose metabolism (subjects 6 and 7, Supplementary Table 1). In four subjects, impairments in both the cardiac noradrenergic and nigrostriatal dopaminergic systems preceded altered cerebral glucose metabolism (subjects 9-11, 13). In subject 8, cardiac noradrenergic denervation and altered cerebral glucose metabolism preceded dopaminergic denervation. In subjects with pathological [123I]MIBG and [123I]FP-CIT-SPECT and suprathreshold baseline PDRP expression, the order in which changes occurred is uncertain (subjects 12, 14-17).

A further point may favor that [18F]FDG-PET shows a PDRP before the [123I]FP-CIT-SPECT becomes reduced: we previously have identified an abnormal iRBD [18F]FDG-PET pattern in a cohort of iRBD patients, even though the minorityof this cohort had dopaminergic deficiencies.4 This iRBD-related pattern overlapped with the PDRP (but was less extensive). It did not include widespread cortical (occipital) hypometabolism as is the case in DLB. In addition it is known that [123I]FP-CIT-SPECT of DLB patients can be initially normal and only become abnormal over time5.

One possible explanation is, that once pathology hits the brainstem (before the substantia nigra in the midbrain), changes in the locus coeruleus and perhaps other brainstem nuclei (i.e. raphe) may already cause brain-wide metabolic changes due to alternations at the network level (for discussion, see 6).

*Age Effect*

As described in the supplementary material of our recent brief report, age of the 32 HC used for PDRP derivation and z-transformation did not correlate with PDRP expression, although a trend was observed 3. In another study7, the PDRP was also not correlated to age in controls but was correlated with age in PD subjects, which perhaps also relates to disease duration. Metabolic decreases have been reported in the parietal cortex in normal aging 8, 9, which may overlap with the PDRP. A more recent study also discusses the age effect on PDRP expression.10 That said, expression of an age-related spatial covariance pattern was shown to be independent from PDRP expression 11, 12.

# **References**

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