



Title	3D neuromelanin-sensitive magnetic resonance imaging with semi-automated volume measurement of the substantia nigra pars compacta for diagnosis of Parkinson's disease
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**ORIGINAL RESEARCH**

**3D Neuromelanin-Sensitive Magnetic Resonance Imaging with  
Semi-Automated Volume Measurement of the Substantia Nigra Pars  
Compacta for Diagnosis of Parkinson's Disease**

## **Abstract**

**Purpose:** Neuromelanin-sensitive MRI has been reported to be used in the diagnosis of Parkinson's disease (PD), which results from loss of dopamine-producing cells in the substantia nigra pars compacta (SNc). In this study, we aimed to apply a 3D turbo field echo (TFE) sequence for neuromelanin-sensitive MRI and to evaluate the diagnostic performance of semi-automated method for measurement of SNc volume in patients with PD.

**Methods:** We examined 18 PD patients and 27 healthy volunteers (control subjects). A 3D TFE technique with off-resonance magnetization transfer pulse was used for neuromelanin-sensitive MRI on a 3T scanner. The SNc volume was semi-automatically measured using a region-growing technique at various thresholds (ranging from 1.66 to 2.48), with the signals measured relative to that for the superior cerebellar peduncle. Receiver operating characteristic (ROC) analysis was performed at all thresholds. Intra-rater reproducibility was evaluated by intraclass correlation coefficient (ICC).

**Results:** The average SNc volume in the PD group was significantly smaller than that in the control group at all the thresholds ( $P < 0.01$ , student  $t$  test). At higher thresholds ( $>2.0$ ), the area under the curve of ROC ( $A_z$ ) increased (0.88). In addition, we observed balanced sensitivity and specificity (0.83 and 0.85, respectively). At lower thresholds, sensitivity tended to increase but specificity reduced in comparison with that at higher thresholds. ICC was larger than 0.9 when the threshold was over 1.86.

**Conclusions:** Our method can distinguish the PD group from the control group with high sensitivity and specificity, especially for early stage of PD.

## **Keywords**

Neuromelanin, MRI, Parkinson's disease, Substantia nigra

## **Introduction**

Parkinson's disease (PD) is a chronic progressive disorder of motor function, and loss of dopamine-producing cells in the substantia nigra pars compacta (SNc) has been reported in this condition [1]. Dopamine-producing cells contain a black pigment known as neuromelanin [2], which acts as a paramagnetic agent on combining with iron [3]. As a result, T1-weighted neuromelanin-sensitive magnetic resonance imaging (MRI) is able to depict tissue that includes neuromelanin in in vivo experiments, and its signal intensity is related to neuromelanin concentration [3].

Today, the diagnosis of PD is mainly made on a clinical basis such as the United Kingdom Parkinson's Disease Society Brain Bank [4], and the role of imaging is limited to exclusion of other brain disorders. The only imaging modality used for the clinical diagnosis of PD is  $^{123}\text{I}$ -metaiodobenzylguanidine (MIBG) myocardial scintigraphy, which is utilized to estimate sympathetic nerve function [5]; however, this technique provides an indirect estimation of brain nerve function only. In contrast, neuromelanin-sensitive MRI can be used as a direct method to measure the volume or concentration of neuromelanin in the SNc, and therefore may be able to distinguish patients with PD from healthy subjects.

There are several studies which report investigation of the SNc using neuromelanin-sensitive MRI [6-8]. However, most of the previous reports used a 2

dimensional (2D) fast spin echo (FSE) sequence, which provides subjective measurements of signal or volume using user-defined region-of-interest (ROI). This is probably because objective or automated measurements using 2D FSE sequence are difficult, based on the fact that the signal intensity obtained from 2D FSE is not homogeneous [9]. We hypothesized that 3 dimensional (3D) acquisition using field echo sequence might be suitable for neuromelanin-sensitive MRI in order to obtain more homogenous signals [10] and objective volume measurements can be achieved.

The aim of this study was to investigate the diagnostic performance of semi-automated measurement of the SNc volume with 3D neuromelanin-sensitive MRI, for the discrimination of PD patients from healthy volunteers.

## **Subjects and Methods**

### ***Subjects***

From October 2011 to April 2012, we prospectively examined PD patients who were diagnosed by the criteria of the United Kingdom Parkinson's Disease Society Brain Bank. The other inclusion criteria were: newly diagnosed or referred to our hospital, and an age of over 20 years old. Subjects who were contraindicated for MRI (e.g., cardiac pacemaker installed) were excluded. Finally, 18 patients were eligible to participate in this study (8 men and 10 women, mean age  $68.8 \pm 6.4$  years). The durations of illness ranged from 3 years to 24 years (median was 7 years). The numbers of patients with Hoehn-Yahr scale 1, 2, 3, 4, and 5 were 1, 8, 6, 2, and 1, respectively.

Twenty-seven volunteers (13 men and 14 women, average age  $65.9 \pm 6.7$  years) were also included as control subjects. A board-certified neurologist (K.S.) confirmed that these control subjects were free of Parkinsonism and any other neurological disorders. All experiments were carried out after obtaining approval of the institutional review board, and written informed consent was obtained from all subjects.

### ***Imaging Protocols***

A 3-T MRI scanner (Achieva, Philips Medical Systems, Best, Netherlands) with a

32 channel head coil was used in this study. In order to obtain neuromelanin-sensitive T1-contrast, we utilized a single-slab 3D turbo field echo (TFE) sequence with the following characteristics: repetition time, 13 ms; echo time, 2.2 ms; flip angle, 20°; turbo field echo (TFE) factor, 59; field of view, 220 mm; matrix size, 320 × 236; slice thickness, 1 mm; slab thickness, 50 mm; and off-resonance multi-shot magnetization transfer (MT) pulse added (flip angle, 600°; offset frequency, 600 Hz). The scanning time was 6 min 55 sec.

### ***Data Processing and Statistical Analysis***

Gaussian-filter ( $2 \times 2$  mm) smoothing was applied to obtained images using Matlab (R2010a, The MathWorks, Natick, Massachusetts, USA) to remove image noise. This process was needed to facilitate connectivities of high signal areas in the SNc in region growing method described below. Next, the signal intensity of 3D neuromelanin-sensitive images was normalized relative to the decussation of the superior cerebellar peduncle, by measuring the signal intensity of the decussation of the superior cerebellar peduncle with circular ROI using a modified version of a freeware program (Perfusion Mismatch Analyzer, version 3.3.0.4) [11]. This process was needed to minimize the difference in signal intensity in the SNc among the subjects. Then, the



SNC volume was semi-automatically measured using the same program, PMA. Region-growing technique was used to define the volume of interest (VOI) of the SNC. Several seed-points were manually defined in the high-intensity area on each slice which reflected the SNC by an experienced MR technologist (K.O.). Then, a 3D VOI was automatically drawn with varying thresholds; the threshold was a value relative to the signal of the superior cerebellar peduncle. The threshold was changed in a batch process (range from 1.66 to 2.48, step of 0.02), and the SNC volume was automatically recorded for each threshold. The total time of whole postprocessing was approximately 10 min.

The average volume of the PD patient group and the control group was compared using Student's t-test. Receiver operating characteristic (ROC) analysis for the distinction between PD patients and normal subjects was also performed at every threshold. The area under the curve (Az) was calculated as well as sensitivity and specificity, which were determined to achieve the maximum value of the Youden index (sensitivity + specificity - 1). This ROC analysis was also performed for early stage of patients (Hoehn-Yahr scale 1 and 2) and advance stage (Hoehn-Yahr scale 3, 4, and 5), respectively.

In order to evaluate intra-rater reproducibility, the process of SNC volume

measurement was performed again by the same technologist after 9 months later.

Intraclass correlation coefficient (ICC) was calculated from these two measurements.

## Results

Neuromelanin-sensitive images at the level of the midbrain showed bilateral high signal intensity areas which reflected the SNc (Fig. 1). The VOI was larger at lower thresholds and became smaller at higher thresholds. When the threshold was around 2.0, the VOI corresponded well to high signal in the SNc, both for PD and control subjects.

The average SNc volume of the PD group was smaller than that of the control group at all thresholds (Fig. 2). When the threshold was greater than 2.1, the volume of the SNc of more than one patient became zero. Therefore, the upper limit of the threshold for further analysis was set to 2.1. The difference in average SNc volume between the PD and control groups was statistically significant for all thresholds ( $P < 0.01$ , Student's t-test, Fig. 3). When the threshold was over 1.8, the  $P$ -value was less than 0.001.

The results of ROC analysis was summarized in Fig. 3. The Az value gradually increased in accordance with threshold increase. When the threshold was larger than 2.0, the maximum value of Az (0.88) was achieved, and sensitivity and specificity were well balanced (0.83 and 0.85, respectively). At lower thresholds, sensitivity tended to be higher but specificity became lower. The highest sensitivity (1.00) was achieved at the thresholds of 1.74, 1.76, and 1.96, while specificity became lower (0.41, 0.41, and 0.63, respectively) at these thresholds. The highest specificity (0.89) was observed at the

threshold of 1.66; however, sensitivity at this threshold became 0.56. The example of volume distribution of SNc at the threshold 2.0 was shown in Fig. 4. Although there were overlaps between PD group and controls group, the sensitivity and specificity was 0.83 and 0.82, respectively. At that threshold, cutoff value was 285 mm<sup>3</sup>. In the separate evaluation of ROC analysis in early and advanced stages of PD patients at threshold 2.0, the sensitivity, specificity, and Az of early stage were 0.89, 0.85, 0.90, respectively, while those of advanced stage were smaller (0.78, 0.63, 0.86, respectively) (Fig. 5).

When the threshold was over 1.86, the ICC was larger than 0.9, which meant good intra-rater reproducibility. The maximum value of ICC was 0.95 at the threshold of 2.06, and the minimum ICC was 0.81 at the threshold of 1.70.

## Discussion

In the present study, we demonstrated feasibility of 3D acquisition in neuromelanin-sensitive MRI and the semi-automated measurement of SNc volume. Nakane et al. have already reported neuromelanin-sensitive MRI with 3D acquisition using MT pulse on 1.5-T [12]. MT pulse is considered to be important for neuromelanin-sensitive MRI in order to obtain high contrast between background tissue and neuromelanin, because the effect of MT pulse is reduced in neuromelanin [12]. However, it is difficult to apply MT pulse with 3-T because of the limitation in the specific absorption ratio (SAR), although 3-T is more suitable than 1.5-T because of high signal-to-noise ratio and prolonged T1. Therefore, we used a TFE sequence with a factor of 59 and centric order of k-space filling. In this case, 59 pairs of radio frequency (RF) pulse and following readout segment were grouped, and MT pulse was added before the first RF pulse of every group. Therefore, the number of MT pulses was significantly reduced compared to the conventional method for MT pulse, which is added before every RF pulse. In addition, centric order of k-space filling enabled us to maintain neuromelanin contrast although the periphery of k-space had less MT effect.

In contrast to 3D, 2D FSE acquisition has been used for most neuromelanin-sensitive MRI [7, 12]. 2D FSE uses many refocusing pulses, which has a

MT effect. Therefore, neuromelanin contrast can be achieved with a 2D FSE sequence. However, there are theoretical disadvantages of 2D acquisition compared with 3D acquisition. Signal intensities of 2D FSE acquisition are not homogenous due to the sensitivity to the radiofrequency (B1) field [8,9], and 2D acquisition generally needs a space gap between slices. From these reasons, even if the SNc area of a slice can be measured by 2D acquisition, accurate measurement of entire SNc volume might be difficult. In addition, longer acquisition time and/or thicker slice thickness is needed to compensate for lower signal-to-noise ratio in 2D sequence. We assume that 3D acquisition can overcome these limitations in 2D acquisition [9].

Degeneration of dopaminergic neurons occurs in the substantia nigra in both PD patients [1] and healthy subjects; however, neuronal degeneration has been reported to be accelerated in PD patients [13]. Therefore, volumetric measurement of the SNc can distinguish PD patients from healthy subjects. Kashihara et al. reported measurement of SNc volume using neuromelanin-sensitive MRI [14]. They reported that the SNc volume of control patients was  $128.4 \text{ mm}^3$  and that of PD patients was  $96.9 \text{ mm}^3$ . These values are much smaller than our results, even at a higher threshold. For example, the SNc volumes of PD patients and controls at a threshold of 2.0 were 215.0 and  $370.3 \text{ mm}^3$ , respectively. These differences might be attributable to differences in spatial

resolution and partial volume averaging. Kashihara et al. used a 2D technique and their slice thickness was 2.5 mm with a 1 mm gap, while our 3D sequence used a 1 mm slice thickness without a gap. 3D measurement of SNc volume has been reported by Menke et al. using driven equilibrium single pulse observation of T1 (DESPOT1), with isotropic data acquisition of 1.1 mm [15]. The SNc volumes of PD and control subjects were reported as 853 and 1006 mm<sup>3</sup>, respectively. These values are much larger than our results at a threshold of 2.0; however, they correspond well to our results of 887.2 and 1103.7 mm<sup>3</sup>, respectively, at a lower threshold of 1.78. In histopathological research, average cell loss, and not volume, of the SNc at the onset of PD symptoms has been reported to be 48% [13]. The volume loss of the SNc calculated from our results was 18%, which is smaller than average cell loss; however, the reduction of SNc volume has been reported to be milder than that of cell loss [13, 14].

We assume that a threshold around 2.0 might be optimal because the VOI corresponded well to the SNc on visual assessment, and high sensitivity, high specificity, and high intra-rater agreement were achieved. In contrast, higher sensitivity can be chosen at the risk of lower specificity, as well as higher specificity and lower sensitivity. Our results suggested that the threshold of the VOI should be smaller if higher sensitivity is desired, and larger if higher specificity is needed. In addition, our method

was more suitable for early stage compared with advanced stage because higher sensitivity and specificity were obtained from early stage compared with advanced stage. Although our method could not discriminate 2 groups perfectly (i.e., there were overlaps in SNc volumes), it could discriminate PD patients from control subjects with almost equal or better performance compared with other imaging methods, such as contrast ratio method using neuromelanin images [7,8], and diffusion-kurtosis imaging [16]. Therefore, our method can be potentially used for clinical diagnosis of PD patients, especially for early stage.

In clinical practice,  $^{123}\text{I}$ -MIBG myocardial scintigraphy is routinely used. It has been reported that this modality could differentiate PD from other neurological diseases mimicking PD [5]. The ratio of average counts per pixel in the heart and mediastinum (H/M ratio) was significantly less than controls ( $P < 0.001$ ). However, this method has the disadvantage of using a radioisotope. In contrast, MRI-based methods have the advantage that they do not involve radiation exposure, and are therefore less invasive. Further study is needed to compare diagnostic performance between these 2 modalities.

Neuromelanin works as a paramagnet when it is combined with ferrous material [3, 6]. Neuromelanin-sensitive MRI may underestimate SNc volume because of ferrous accumulation in the SNc of PD patients [17]. Correction of ferrous accumulation might



be needed for more accurate estimation of SNc volume and increased sensitivity and specificity, as the signal intensity obtained from 3D field echo acquisition is affected by ferrous accumulation in the SNc in comparison with 2D FSE acquisition. There are 2 potential methods to correct ferrous accumulation; one uses the T2\* value obtained from multi-echo field echo sequence, and the other involves quantification of phase shift relative to the surrounding normal tissue. These potential improvements in neuromelanin-sensitive MRI may allow more accurate estimation of SNc volume.

In the current study, semi-automated measurement of SNc volume was feasible, with only several clicks on the SNc (for seeding points) and decussation of the superior cerebellar peduncle (for signal normalization) required. Although intra-rater reproducibility of this method was good ( $> 0.9$ ), these steps were operator-dependent. Development of an algorithm that allows automatic determination of seed points and signal normalization would be needed for fully-automated measurement of SNc volume and for the reduction of processing time.

There are several limitations in this study. First, we used region-growing technique for semi-automated measurement of SNc volume; however, the processes of determination of seed points and normalization of signal intensity could not be automated. Second, clinical data such as period of illness, Hoehn-Yahr stage, severity of

illness, and H/M ratio obtained from  $^{123}\text{I}$ -MIBG were not evaluated in this study. Further evaluation is needed to elucidate the relationships between these factors and SNc volume. Third, the number of patients was relatively small. Further study is needed for sub-group analysis with different stage of disease in larger patient population. Finally, although we measured SNc volume, signal intensity was not determined. There have been several studies which reported loss of signal intensity in the SNc in PD patients. Further study is needed to compare the efficacy of signal intensity measurement and volume measurement in the SNc.

## **Conclusion**

A semi-automated measurement of SNc volume could distinguish PD patients from healthy control subjects with high sensitivity and specificity, although there were overlaps between these groups. This method was more effective in early stage of PD than advanced stage.

## **Conflict of Interest**

We declare that we have no conflict of interest.

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## Figure Captions

### **Fig. 1** Neuromelanin-sensitive MRI at the level of the midbrain

The SNc is seen as a high intensity area (arrows) in a control subject (a ~ e) and a PD patient (f ~ j). The control subject was a 69-year-old male, and the PD patient was a 64-year-old female with a Hoehn-Yahr score of 4. The VOIs at a threshold of 1.8 (b, g), 1.9 (c, h), 2.0 (d, i), and 2.1 (e, j) are shown as red pixels in the control (b ~ e) and PD (g ~ j) subjects. The size of the VOI decreases with the increase of threshold. Note that the volume of the SNc is smaller in the PD patient than in the control subject. Seed points are also shown as blue dots.

### **Figure 2.** SNc volume at the various thresholds of VOI.

SNc volume is larger in PD group (○) than in control group (●) at all the thresholds.

The overlap of these two groups becomes smaller at higher threshold.

### **Figure 3.** Summary of ROC analysis.

The area under the curve ( $A_z$ , ●) increases with the increase of threshold. When the threshold is higher than 2.0,  $A_z$  becomes maximum, and the sensitivity ( $\Delta$ ) and specificity (○) are balanced. Youden index (■) is also shown.

**Figure 4.** The distribution of SNc volume.

SNc volumes of PD patients (◆) and controls (◇) at the threshold 2.0 are shown. The dotted line represents cut-off value of 285 mm<sup>3</sup>, which is determined by ROC analysis. There are overlaps between two groups; however, the sensitivity and specificity were 0.83 and 0.82, respectively.

**Figure 5.** ROC of 2 groups and total at threshold 2.0.

The result of ROC analysis of early stage of PD (○), advanced stage (●), and total of PD (×).



Figure 1

a



b



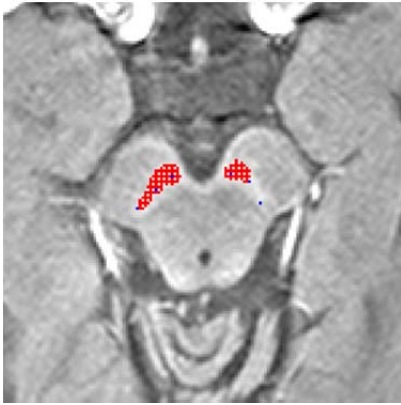
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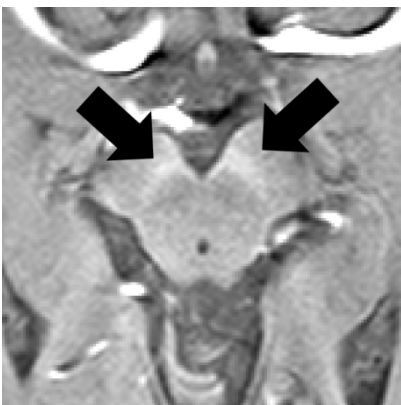
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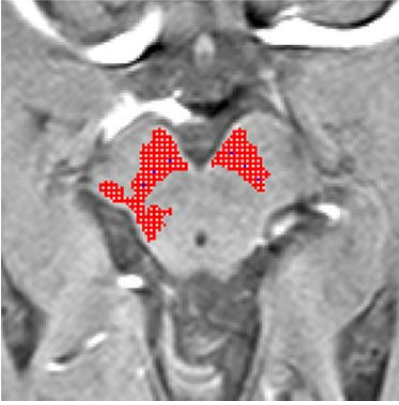
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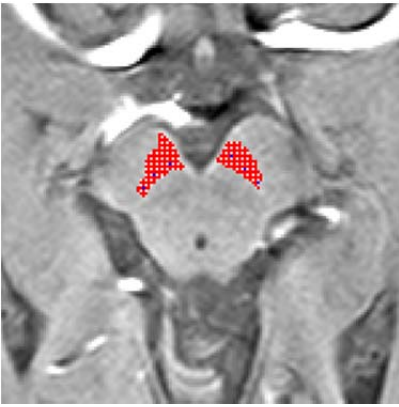
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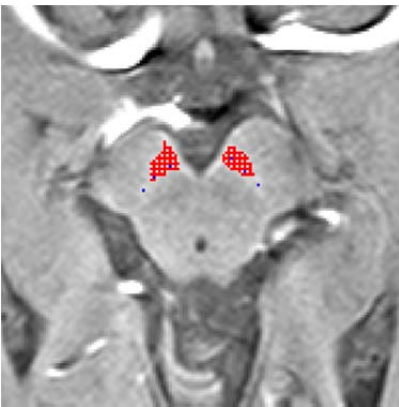
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h



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j

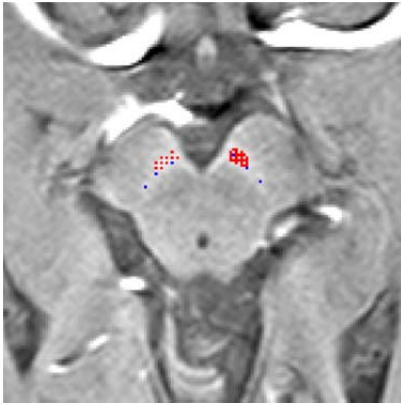


Figure2

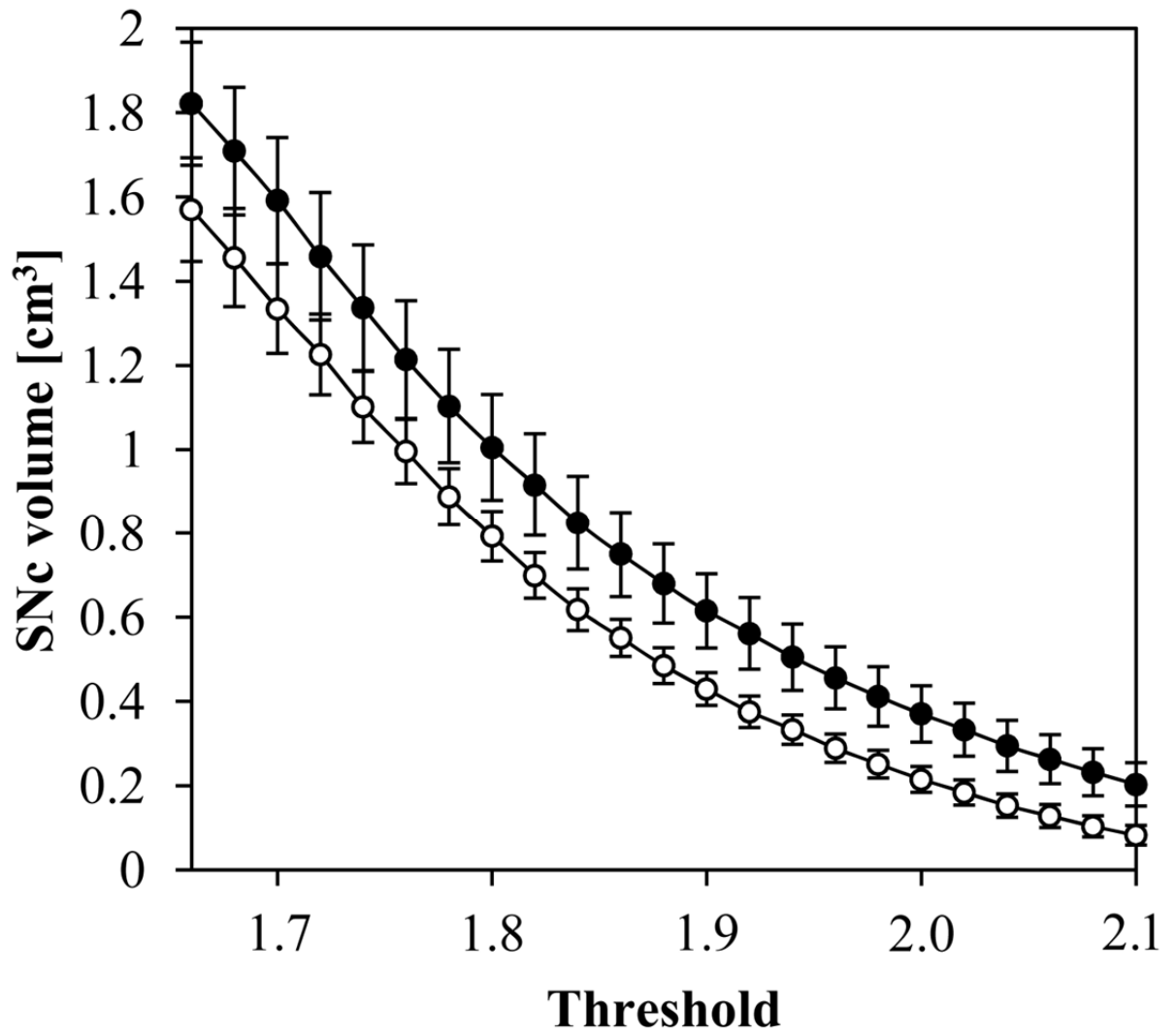


Figure3

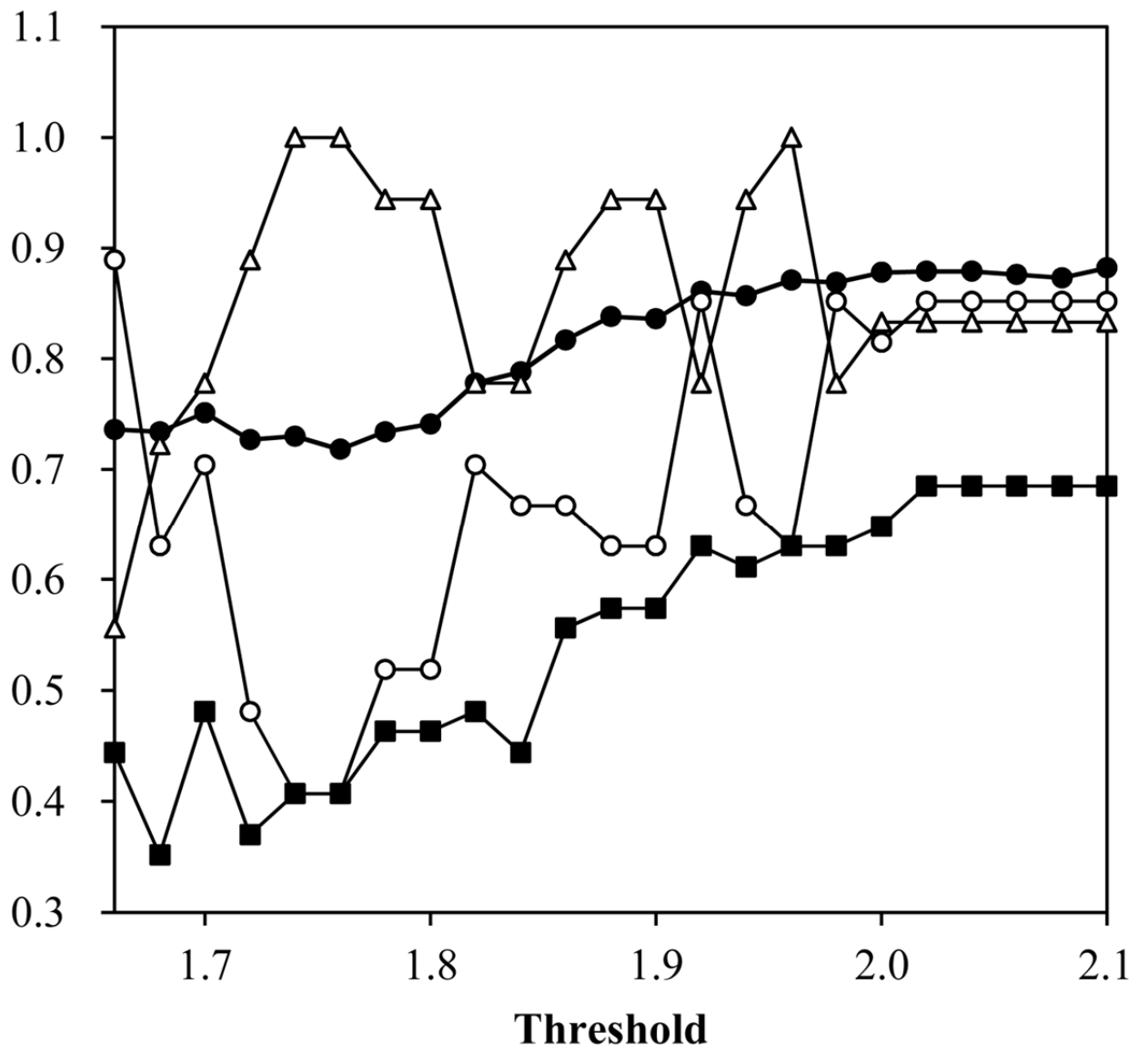


Figure 4

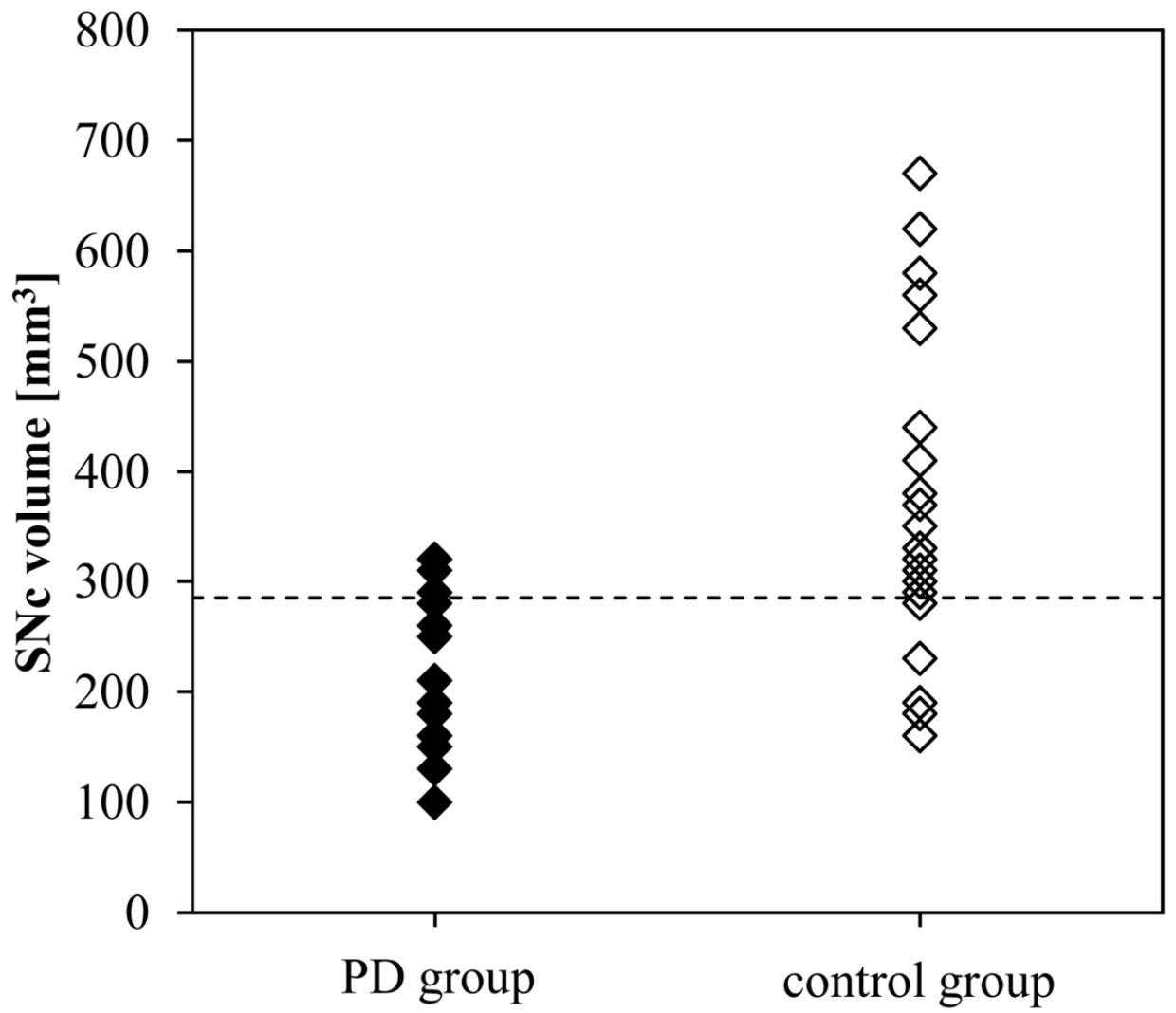


Figure 5

