Diagnosing a tremor disorder accurately is challenging as similar clinical entities can be caused by different diseases. Therefore, in the absence of biomarkers, misdiagnoses between tremor in Parkinson’s disease (PD), tremor in dystonia (TiD), and essential tremor (ET) frequently occur. Ioflupane-based single photon emission computed tomography (123I-FP-CIT DAT-SPECT) can assist in differentiating PD from ET and TiD by detecting a presynaptic dopaminergic deficit. In recent years, quantitative magnet resonance imaging (MRI) markers have been studied as widely available, noninvasive, radiation-free alternative. R2* relaxation rate mapping allows to measure iron in a refined and quantitative manner. R2* values in the substantia nigra (SN) have been shown to be increased in PD, but have not yet been investigated in TiD and are inconclusive in ET, in whom 1 study has reported increased iron in the globus pallidus (GP).

The aim of this study was to investigate R2* relaxation rates in the SN of patients with tremor-dominant PD, TiD, and ET and to compare the results to age-matched controls. A second goal was to evaluate the contribution of nigral R2* as a neuroimaging biomarker in the diagnosis of tremor-dominant PD and for the differential diagnosis of common tremor disorders.

**Participants and Methods**

**Participants**

Patients were recruited as participants of the single-center cohort study titled “Prospective Movement Disorders Registry Graz” at the Department of Neurology, Medical University Graz, Austria. Inclusion criteria were a clinical diagnosis of tremor-dominant PD, TiD, or ET following the Queen Square Brain Bank diagnostic criteria for PD and the criteria of the consensus statement of the Movement Disorder Society on tremor for ET and TiD. Tremor-dominant PD was defined as a MDS-UPDRS resting tremor score of ≥2 for at least 1 hand. All patients had an upper-limb tremor and an 123I-FP-CIT DAT-SPECT had revealed normal (TiD, ET) or abnormal (PD) results within the
previous 12 months. Exclusion criteria were significant cognitive impairment (Mini Mental State Examination < 24), secondary or atypical parkinsonian disorders, a history of tremorgenic drugs use, and structural abnormalities on routine MRI. Age-matched healthy controls without first-degree relatives with any movement disorder were recruited from an ongoing community-dwelling aging cohort.12

All patients, 40 tremor-dominant PD, 15 age-matched TiD (3 dystonic tremor, 12 tremor associated with dystonia) and 25 age-matched ET and 25 age-matched controls underwent comprehensive neurologic examination and quantitative MRI of the brain.

MRI Acquisition

MRI was performed on a 3 T whole-body scanner (TimTrio; Siemens Healthcare, Erlangen, Germany) and included conventional imaging and magnetization transfer imaging (details in Supplementary Data).

Image Processing and Analysis

The R2* image processing has been described previously.7 The SN was segmented manually by 1 (all cases) or 2 (20 cases; interclass correlation coefficient 0.970) experienced blinded raters. Mask volumes, mean R2* rate constants, and the respective standard deviations were calculated for the SN and GP (details in Supplementary Data).

Statistical Methods

We compared the volumes of the SN masks and the means of R2* values in the SN and GP between all 4 groups using analysis of variance. Next, we compared the means of R2* between 6 pairs of 2 groups using independent t tests and corrected for multiple comparisons using the false discovery rate.13 P values < 0.05 were considered significant. To evaluate the discriminatory power of nigral R2*, we performed a receiver operating characteristics (ROC) analysis, selected an optimal R2* cutoff value, and compared the frequency of normal and abnormal test results by Fisher’s exact (2-sided) test and obtained sensitivity and specificity (details in Supplementary Data).

### Results

Demographic and clinical characteristics are listed in Table 1. The volumes of the SN masks were 289.9 ± 107.1 mm³ in tremor-dominant PD, 313.4 ± 92.5 mm³ in TiD, 286.8 ± 101.7 mm³ in ET, and 331.3 ± 86.8 mm³ in healthy controls (P = 0.319).

Mean R2* relaxation rates of the SN were 34.1 ± 5.7 s⁻¹ in tremor-dominant PD, 30.0 ± 3.9 s⁻¹ in TiD, 30.6 ± 4.8 s⁻¹ in ET, and 30.0 ± 2.8 s⁻¹ in healthy controls and significantly different over all groups (P = 0.002).

Every participant’s individual nigral R2* values are shown in Figure 1A. Significant higher R2* values in the SN were found in tremor-dominant PD when compared with the TiD, ET, and control groups (P < 0.023), respectively. No significant differences were found between ET and TiD, ET and controls, and TiD and controls.

A ROC analysis of nigral R2* (considering as target a diagnosis of tremor-dominant PD over healthy controls) afforded an area under curve of 0.721 (95% confidence interval, 0.598–0.844; see Fig. 1B). The optimal R2* threshold in this dataset was 31.15. Mean nigral R2* values ≥ 31.15 were indicative of tremor-dominant PD (abnormal test result), and R2* values < 31.15 indicative of healthy controls (normal test result). The diagnostic performance of nigral R2* in this cohort was modest with a sensitivity of 67.5%, specificity of 72.0% (P = 0.002). Comparing abnormal and normal

### Table 1. Clinical and demographic data of the study participants

| Variable          | PD (n = 40) | TID (n = 15) | ET (n = 25) | Controls (n = 25) | P value |
|-------------------|------------|-------------|------------|------------------|--------|
| Age, y            | 65.06 ± 10.60 | 62.29 ± 8.18 | 65.80 ± 12.82 | 64.60 ± 11 | 0.799 |
| Sex, woman, N     | 17         | 7           | 10         | 12               | 0.211 |
| Disease duration, y | 5.04 (2.33–6.81) | 13.50 (6.58–31.08) | 10.58 (4.79–19.29) | –          | <0.001* |
| H&Y               | 2.05 ± 0.51 | –           | –          | –                | –      |
| MDS-UPDRS III     | 37.10 ± 15.16 | –           | –          | –                | –      |
| MDS-UPDRS total   | 55.64 ± 24.01 | –           | –          | –                | –      |
| FTMTRS            | 19.72 ± 16.75 | 26.47 ± 21.49 | 26.56 ± 15.91 | –              | 0.230 |
| BMF/DS            | 4 ± 2.10   | –           | –          | –                | –      |
| Rest tremor b     | 4.02 ± 2.33 | 1.46 ± 2.29 | 2.16 ± 1.43 | –                | <0.001* |
| Postural tremor b | 2.10 ± 1.17 | 2.33 ± 1.49 | 2.60 ± 1.15 | –                | 0.286 |
| Kinetic tremor b  | 1.9 ± 1.31 | 2.66 ± 1.75 | 2.76 ± 1.66 | –                | 0.057 |

Group comparisons were done using analysis of variance. PD, tremor-dominant Parkinson disease; TID, tremor in dystonia; ET, essential tremor; H&Y, Hoehn & Yahr scale; MDS-UPDRS total, Movement Disorder Society sponsored revision of the Unified Parkinson’s Disease Rating Scale consisting of 4 parts; MDS-UPDRS III, third part of the MDS-UPDRS; FTMTRS, Fahn-Tolosa Marin Tremor Scale; BMF, Burk-Fahn-Marsden Scale.

b Median (range); all other values are given as mean ± standard deviation.

b Calculated from the MDS-UPDRS III.

*P values < 0.05 were considered significant.
test results between tremor-dominant-PD and a merged group of ET and TiD using the same R2* cut-off score yielded a test sensitivity of 67.5% and a specificity of 60% ($P = 0.024$). The false positives were 10 patients with ET and 6 patients with TiD. No significant difference was found regarding the frequency of abnormal and normal test results in ET and TiD compared to healthy controls ($P = 0.426$). The positive and negative predictive values of R2* were 79% and 58% between tremor-dominant PD and healthy controls and 63% and 65% between tremor-dominant PD and the merged group of ET and TiD.

Mean R2* relaxation rates of the GP were $38.6 \pm 5.7$ (s$^{-1}$) in tremor-dominant PD, $38.9 \pm 3.9$ (s$^{-1}$) in TiD, $39.5 \pm 5.0$ (s$^{-1}$) in ET, and $36.9 \pm 3.1$ (s$^{-1}$) in healthy controls and did not differ across all groups ($P = 0.352$). A nonsignificant trend was found for a difference between ET and controls ($P = 0.052$, uncorrected), whereas all other 2-group comparisons revealed no difference ($P > 0.1$).

In the PD group, we found a significant correlation for nigral R2* and total MDS-UPDRS and disease duration (see Supplementary Table 2). PD patients with normal R2* values had a significantly shorter disease duration, lower MDS-UPDRS total scores, and less symptoms associated with restless legs syndrome (RLS) when compared with the group with abnormal R2* values (see Supplementary Table 3). The latter was indicated by a positive answer to item 26 of the Nonmotor Symptoms Questionnaire.\textsuperscript{14} 

**Discussion**

In this study, we showed for the first time that the iron content in the SN assessed by R2* is significantly increased in the tremor-dominant PD motor phenotype and is significantly higher in tremor-dominant PD when compared with TiD, ET, and healthy controls. Nigral R2* values in TiD and ET did not differ significantly from healthy controls, suggesting a normal iron load.

So far only 1 study had analyzed the ability of nigral R2* to classify healthy individuals and patients with PD using the ROC, which displayed an area under the curve of 0.67.\textsuperscript{6} In our study, ROC analysis displayed an area under the curve of 0.721. After determining the optimal R2* threshold to discriminate PD from healthy controls, we found a modest test sensitivity and specificity of 67.5% and 72%, respectively. False negative test results were more common in PD patients with a disease duration less than 5 years (10/20 patients) compared to those with longer standing disease (3/20 patients).

Using the same threshold for an abnormal or normal test result, we found a test sensitivity of 67.5% and a specificity of 60%, with a considerable overlap between tremor-dominant PD and the other tremor disorders, showing increased nigral iron accumulation in 40% of our ET and TiD patients, respectively. Although abnormal test results were more common in ET and TiD (40%) than in healthy controls (28%), this difference was not significant. Only 1 study has so far investigated brain iron content in patients with ET when compared with healthy controls and found increased values in the GP.\textsuperscript{8} We found a trend comparing mean R2* values in the GP between ET and controls ($P = 0.052$). However, we did not find significant differences comparing all four groups and other pairs of groups, which argues against an additional contribution of R2* in the GP to the diagnostic accuracy in the discrimination of tremor disorders.
Previous MRI studies have shown reduced nigral iron in patients with RLS. We investigated if an association of RLS and PD may explain normal R2* values in some patients with PD. Contrary to this assumption, all patients with PD and RLS (n = 10) had abnormal R2* values. This finding favors the argument that RLS could represent a secondary condition of PD.

We consider the cross-sectional design, the small numbers of participants, especially in the TiD group, and the lack of an independent validation cohort as limitations of this study. As age has been described in previous studies as influencer of R2*, we used age-matched patients. Unfortunately, our cohort did not allow matching for age and disease duration, which is a major limitation of the study. Another limitation is that we did not separate the SN into its pars compacta and pars reticulata subregions. Neuronal loss from the SN pars compacta has been reported to be particularly related to the clinical features of PD. However, even with improved resolution on 3 Tesla MRI, defining the border between the subdivisions is difficult and to avoid this controversy we studied the total SN.

Iron accumulation follows an exponential saturation function with only little changes after the fourth to fifth decade. Nevertheless, defining age-dependent nigral R2* cut-off values might help to improve its diagnostic properties. Comparative studies of R2* and quantitative susceptibility mapping suggested that quantitative susceptibility mapping had higher sensitivity for displaying PD-related changes in the SN and correlated better with clinical parameters than R2*. There is evidence that neuromelanin-sensitive MRI, resting-state functional MRI, or evaluation of loss of nigral hyperintensity in dorsolateral parts of the SN on iron sensitive sequences might be helpful for the discrimination of PD from controls. The previously mentioned MRI techniques alone or in combination may also be helpful for the discrimination of tremor-dominant PD from TiD and ET and should be investigated in future studies.

In conclusion, our study revealed a significantly increased nigral iron content in tremor-dominant PD and normal iron content in TiD and ET. However, the low diagnostic accuracy argues against the usefulness of nigral R2* as a single neuroimaging biomarker for diagnosing tremor-dominant PD and its differentiation from TiD and ET.

References

1. Bhataia KP, Bain P, Bajaj N, et al. Consensus statement on the classification of tremors. from the task force on tremor of the international parkinson and movement disorder society. Mov Disord 2018; 33:75-87.

2. Chen W, Hopfner F, Becktepe JS, Deuschl G. Rest tremor revisited: Parkinson’s disease and other disorders. Tranl Neurodegener 2017; 6:16-017-0086-e, eCollection 2017.

3. Kagi G, Bhataia KP, Tolosa E. The role of DAT-SPECT in movement disorders. J Neurol Neurosurg Psychiatry 2010;81:5-12.

4. Lehericy S, Vaillancourt DE, Seppi K, et al. The role of high-field magnetic resonance imaging in parkinsonian disorders: pushing the boundaries forward. Mov Disord 2017;32:510-525.

5. Ulla M, Bonny JM, Ouchchane I, Rieu I, Claibse B, Durfi F. Is R2* a new MRI biomarker for the progression of parkinson’s disease? A longitudinal follow-up. PLoS One 2013;8:e57904.

6. Barbosa JH, Santos AC, Tumas V, et al. Quantifying brain iron deposition in patients with parkinson’s disease using quantitative susceptibility mapping, R2 and R2*. Magn Reson Imaging 2015;33: 559-565.

7. Langkammer C, Pirramer L, Seiler S, et al. Quantitative susceptibility mapping in parkinson’s disease. PLoS One 2016;11:e0162460.

8. Novellino F, Cherubini A, Chiriaco C, et al. Brain iron deposition in essential tremor: a quantitative 3-tesla magnetic resonance imaging study. Mov Disord 2013;28:196-200.

9. Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic parkinson’s disease. J Neurol Neurosurg Psychiatry 1998;51:745-752.

10. Goetz CG, Fahn S, Martinez-Martin P, et al. Movement disorder society-sponsored revision of the unified parkinson’s disease rating scale (MDS-UPDRS): process, format, and clinimetric testing plan. Mov Disord 2007;22:41-47.

11. Helmich RC, Janssen MJ, Oyen WJ, Bloem BR, Toni I. Pallidal dysfunction drives a cerebellothalamic circuit into parkinson tremor. Ann Neurol 2011;69:269-281.

12. Schmidt R, Enzinger C, Ropele S, Schmidt H, Fazekas F, Austrian Stroke Prevention Study. Progression of cerebral white matter lesions: 6-year results of the austrian stroke prevention study. Lancet 2003;361:2046-2048.

13. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a pracitical and powerful approach to multiple testing. J Stat Soc Series B Stat Methodol 1995;57:289-300.

14. Caudilli HR, Martinez-Martin P, Schapira AH, et al. International multicenter pilot study of the first comprehensive self-completed nonmotor symptoms questionnaire for parkinson’s disease: the NMSQuest study. Mov Disord 2006;21:916-923.

15. Ferini-Strambi L, Carli G, Casoni F, Galbiati A. Restless legs syndrome and parkinson disease: a causal relationship between the two disorders? Front Neurol 2018;9:551.

16. Ropele S, Wartjes MP, Langkammer C, et al. Multicenter R2* mapping in the healthy brain. Magn Reson Med 2014;71:1103-1107.

17. Fearnley JM, Lees AJ. Ageing and parkinson’s disease: Substantia nigra regional selectivity. Brain 1991;114(Pt 5):2283-2301.

18. Gorell JM, Ordidge RJ, Brown GG, Deniau JC, Buderer NM, Helpern JA. Increased iron-related MRI contrast in the substantia nigra in parkinson’s disease. Neurology 1995;45:1138-1143.

19. Du G, Liu T, Lewis MM, et al. Quantitative susceptibility mapping of the midbrain in parkinson’s disease. Mov Disord 2016;31: 317-324.

20. Heim B, Krimer F, De Marzi R, Seppi K. Magnetic resonance imaging for the diagnosis of parkinson’s disease. J Neural Transm (Vienna) 2017;124:915-964.

Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.