Original Paper

Application of Skin Gas GC/MS Analysis for Prediction of the Severity Scale of Parkinson’s Disease

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Abstract
Parkinson’s disease (PD) is diagnosed by neurological examinations, as well as by scintigraphy for the dopamine transporter and metaiodobenzylguanidine (MIBG). We studied possible application of the skin gas in diagnosis of PD. We analyzed chemical substances emanated from the skin by gas chromatograph/mass spectrometer (GC/MS) after on-line-pre-concentrator. We analyzed the skin gas in 61 PD patients and 61 controls. The GC/MS chromatograms were sectionalized every 30 sec. The retention time drift was shifted every 5 sec, and a similarity coefficient (Z score) between a reference chromatogram and each shifted chromatogram was calculated. Chromatograms with high Z scores were excluded from our analysis. Models were made with partial least square (PLS), support vector machine (SVM), and support vector regression (SVR) analyses. PLS modeling to predict the Unified Parkinson’s Disease Rating Scale part 3 (UPDRS3), representing motor deficits in PD, with all the detected mass numbers yielded 50 mass numbers with high PLS coefficients. Among the 50 mass numbers, nine mass numbers (m/e 48, 63, 67, 70, 81, 93, 96, 104, and 105) had dependable signal-to-noise ratios. We then generated an SVM model to differentiate PD and controls. Our SVM model had a sensitivity of 90.2% and a specificity of 85.2% by leave-one-out cross-validation (LOOCV) analysis. We next generated an SVR model to predict UPDRS3 with the nine mass numbers, and obtained a Pearson’s correlation coefficient of 0.834. LOOCV analysis of our SVR model similarly gave rise to a correlation coefficient of 0.710. We propose that chemical substances in the skin gas potentially serve as biomarkers for PD.

Keywords: Parkinson’s disease; Skin gas; GC/MS; Sectional chromatogram; Retention time drift; The Unified Parkinson’s Disease Rating Scale (UPDRS)

1. Introduction
Gas emanated from the skin is composed of a diverse array of organic chemical substances, such as ammonia, ethanol, acetone, toluene, acetaldehyde, limonene to name a few [1,2]. As chemical substances in the breath and the sweat are specifically changed in pathological conditions, the skin gas components are also expected to serve as biomarkers representing pathological conditions of the human body. For example, we previously reported that acetone in the skin gas is elevated in diabetes mellitus especially in diabetic ketoacidosis [3]. Parkinson’s disease (PD) is a neurodegenerative disorder.
characterized by motor and non-motor symptoms, and is caused by abnormal aggregation of α-synuclein (Lewy body) especially in dopaminergic neurons in the substantia nigra in the midbrain. Diagnosis of PD is based on neurological features and auxiliary laboratory findings including the DaTscan scintigraphy for evaluating the dopamine transporter at the dopaminergic nerve terminals in the brain [4] and the metaiodobenzylguanidine (MIBG) scintigraphy for evaluating the sympathetic nerve terminals in the heart [5].

In PD, urinary 8-hydroxydeoxyguanosine (8-OHdG), a marker for oxidative stress against nucleic acids, was increased [6]. We also reported that increased urinary 8-OHdG in PD was associated with hallucination but not with dementia [7]. Serum biomarkers in PD have also been analyzed by mass spectrometry. In PD, serum levels of tryptophan, caffeine, bilirubin, and ergothioneine were lower than controls, and serum levels of levodopa and biliverdin were higher than controls [8]. The same group later reported that serum levels of caffeine and nine of its downstream metabolites were decreased, which was irrelevant to total caffeine intake or disease severity [9]. We also reported by mass spectrometry that the ratio of serum tyrosine to phenylalanine was less than 0.82 in 49% of PD patients, but in none of controls [10]. Trivedi et al. recently reported that perillic aldehyde and eicosane were increased in sebum samples collected from the upper back in PD [11].

In this communication, we collected skin gas samples from PD and controls, and obtained GC/MS chromatographic data [12-15]. We also evaluated the severity of PD using the Unified Parkinson’s Disease Rating Scale (UPDRS) [16]. We employed the partial least square (PLS) regression analysis and the support vector machine (SVM) modeling with a Library for Support Vector Machines (LIBSVM) [17] to differentiate PD and controls. PLS regression analysis showed that chromatograms with retention time from 200 to 400 sec contributed to predict the severity of motor deficits in PD. The retention time of GC/MS chromatograms that were sectionalized every 30 sec was shifted every 5 sec, and chromatograms with low similarity coefficients (high Z scores) were excluded from our analysis. SVM modeling showed that chemical substances with nine mass numbers differentiated PD and controls with a sensitivity of 90.2% and a specificity of 85.2%. Similarly, SVR modeling showed that the same nine mass numbers predicted the severity of motor deficits in PD with a Pearson’s correlation coefficient of 0.834.

2. Subjects and methods

2.1. Subjects

All human studies were approved by the Ethical Review Committee of the Nagoya University Graduate School of Medicine and Graduate School of Pharmaceutical Sciences, the University of Tokyo. The study was registered in the clinical trial registry UMIN (UMIN000019082). Appropriate informed consents were obtained from all PD patients and healthy controls. We enrolled 61 PD patients and 61 controls in the study. Severity of PD were evaluated by the Unified Parkinson’s Disease Rating Scale (UPDRS) [1]. Demographic features of the examinees are summarized in Table 1.

2.2. Skin gas sampling

The skin gas samples were obtained from the right hand in an air-conditioned room at 26°C, but the ambient humidity was not controlled. We first instructed the subjects to thoroughly wash their hands with running water for 1 min. The right hand was sprayed with 1% ethanol, and lightly wiped with KimWipes (Kimberly-Clark, Irving, TX, USA). The right hand was then sprayed with distilled water, and lightly wiped again with KimWipes. The right hand was dried in the room air for 10 min. A skin gas collection bag was inserted with the right hand, and was lightly sealed at the wrist not to affect the blood flow. The gas inside the bag was first sucked away lightly, and then the bag was filled with 130-ml clean air (grade 1) in a gas cylinder. This process was repeated again. The subjects were instructed to stay still for 10 min. The gas in the bag (~120 ml) was transferred to a sampling bag. Both the collection and sampling bags were made of a polytetrafluoroethylene sheet.

2.3. Mass spectrometry of skin gas

The skin gas was subjected to gas chromatograph/mass spectrometry. The skin gas was subjected to gas chromatograph/mass spectrometry.
spectrometer (GC/MS, Shimadzu GC/MS QP 2010, Kyoto, Japan) following an on-line pre-concentrator (Cooled Gas Pre-concentrator, NIT-P, Pico-device, Nagoya, Japan). The core of the pre-concentrator was a small cryostat (outer dimensions of 20 x 20 x 60 mm with an inner volume of 0.05 to 0.10 ml). The cryostat was cooled with partly evaporated liquid nitrogen. The pre-concentrator was simultaneously heated by pulsed direct current to keep the temperature at -100˚C. The skin gas (25 ml) was introduced into the pre-concentrator for 15 sec, and then the cryostat was heated up to 120˚C instantly using a direct current of 5 ampere. The skin gas was thus concentrated ~250 times, and was introduced into GC/MS on-line.

The GC/MS conditions were as follows: DB-WAXetr capillary column (Shimadzu) with 0.32 mm in inner diameter, 30 meters in length, and 1 µm in film thickness. The temperature was initially set to 40˚C for 3 min, gradually increased by 20˚C/min up to 180˚C, and held at 180˚C for 3.5 min. The initial cut period due to solvent elution was 120 sec.

2.4. Selection of retention time windows and mass numbers with statistical analysis

The retention time was sectionalized every 30 sec starting from 120 sec. The partial least square (PLS) regression analysis to predict motor deficits evaluated by UPDRS3 in 61 PD patients was performed using all mass numbers from m/e 48 to 200 with an R package. We found that the retention time from 200 to 400 sec best contributed to predict the UPDRS3 scores. PLS analysis also showed that 50 mass numbers had highest coefficients. We then selected nine mass numbers with high signal-to-noise ratios from the 50 mass numbers by visual inspection.

2.5. Selection of reliable chromatographic data with the help of statistical analysis

To examine the retention time drift, we next compared GC/MS chromatograms of each individual with those of a putative reference person. The GC/MS chromatograms were sectionalized every 30 sec. The sectionalized GC/MS chromatograms were checked concerning retention time drift with a reference chromatogram, as follows:

The GC/MS chromatograms were sectionalized every 30 sec. The retention time drift was shifted every 5 sec, and we calculated a similarity coefficient (Z score) of the nine mass numbers between a reference chromatogram and each shifted chromatogram. Chromatograms of 41 PD patients had Z scores within the range of -0.8 to 0.8, and were included in our analysis.

We next generated support vector machine (SVM) models with LIBSVM [2] to differentiate PD and controls using the nine mass numbers. We also generated support vector regression (SVR) models with LIBSVM to predict UPDRS3 scores in PD patients using the nine mass numbers. Both SVM and SVR models were subjected to the leave-one-out cross-validation analysis, in which a single individual (PD or control) was excluded from making a model, and the excluded individual was used to test the validity of the generated model.

2.6. Candidates of chemical substances in the skin gas associated with severity of PD

Candidate chemical substances that were potentially associated with severity of PD were estimated from GC/MS of the 41 PD patients. The retention time was sectionalized by a sliding window of 60 sec that started every 30 sec. The first window started at 120 sec. PLS analysis to predict UPDRS3 scores was performed using the nine mass numbers in each sectionalized time window. We found that

| Mass Number | Coefficient | Mass Number | Coefficient |
|-------------|-------------|-------------|-------------|
| 87          | 31885       | 99          | -35465      |
| 102         | 26708       | *105        | -32281      |
| 53          | 25296       | 54          | -25529      |
| 89          | 22321       | 69          | -15851      |
| 101         | 22062       | 82          | -14638      |
| &93         | 18780       | 94          | -13961      |
| 97          | 18622       | *67         | -12506      |
| &96         | 18261       | 107         | -11106      |
| 68          | 16170       | 109         | -8887       |
| 103         | 14995       | 61          | -7766       |
| &81         | 14337       | 64          | -7631       |
| 98          | 14171       | 76          | -7599       |
| 77          | 10634       | 52          | -7565       |
| 80          | 10058       | 65          | -7337       |
| 88          | 8950        | 62          | -7332       |
| 50          | 7128        | *63         | -6723       |
| &70         | 6929        | 86          | -6609       |
| 90          | 5993        | 66          | -6308       |
| 72          | 4878        | 83          | -5657       |
| 100         | 4507        | 95          | -5631       |
| 108         | 3485        | *104        | -5372       |
| 71          | 3083        | 106         | -4670       |
| 85          | 2569        | 74          | -3997       |
| 51          | 1506        | *48         | -3640       |
| 75          | 363         | 60          | -3422       |

*Nine mass numbers selected for the downstream SVM and SVR modeling.

UPDRS3 scores in PD patients using the nine mass numbers. Both SVM and SVR models were subjected to the leave-one-out cross-validation analysis, in which a single individual (PD or control) was excluded from making a model, and the excluded individual was used to test the validity of the generated model.
four PLS coefficients constituted a group of positive effect sizes (180-240 sec, 330-390 sec, 570-630 sec, and 510-570 sec in descending order of coefficients) and that two PLS coefficients constituted a group of negative effect sizes (630-690 sec and 450-540 sec in descending order).

3. Results

3.1. Extraction of nine mass numbers to predict UPDRS3 scores

We predicted UPDRS3 scores using all the mass numbers ranging from m/e 48 to 200 in 61 PD patients by PLS modeling. We visually scrutinized chromatograms of 50 mass numbers with the highest positive and negative coefficients (Table 2) in the range of m/e 48 to 200, and extracted nine mass numbers with high signal-to-noise ratios. The best selected nine mass numbers were m/e 48, 63, 67, 70, 81, 93, 96, 104, and 105 (Table 2 and Fig. 1).

3.2. SVM model to differentiate PD patients from healthy controls

To examine the validity of the nine extracted mass numbers, we generated SVM models to differentiate 61 PD patients and 61 healthy controls. Validity was corroborated by the leave-one-out cross validation (LOOCV) analysis. The cross-validation revealed that 55 out of 61 PD patients were classified into PD (sensitivity = 90.2%). Similarly, 52 out of 61 healthy controls were classified into controls (specificity = 85.2%). These values also yielded a positive predictive value (PPV) of 85.9% (= 55/64) and a negative predictive value (NPV) of 89.7% (= 52/58). The nine mass numbers to predict UPDRS3 scores were thus able to efficiently differentiate PD patients and healthy controls.

3.3. Prediction of UPDRS scores using the nine mass numbers

We next generated an SVR model to predict UPDRS3 scores using the nine mass numbers. We used 41 PD patients who had Z scores of the nine mass numbers all within the range between -0.8 to 0.8 (see Materials and Methods). To confirm that UPDRS scores can be predicted by SVR modeling using the nine mass numbers, we validated our strategy by LOOCV. The cross-validation revealed that SVR was able to make descent models with a Pearson’s correlation coefficient of 0.710 (Fig. 2A). We next generated a global SVM model using the nine mass numbers of the 41 PD patients, and obtained a correlation coefficient of 0.834 (Fig. 2B).

3.4. PD drugs are unlikely to have an effect on the nine mass numbers

Among the 41 patients shown in Fig. 2, 36 patients were taking L-Dopa (Table 1). UPDRS3 scores were weakly correlated with plasma L-Dopa concentration (correlation coefficient, R = 0.501), but not with L-Dopa dose (R = 0.135) or L-Dopa-equivalent daily dose that included other anti-PD drugs (R = 0.319). We found that none of the nine mass numbers were correlated with these L-Dopa-related features (Table 3). We also calculated Z scores of each mass number in the 41 patients. We compared the Z scores by the presence or absence of each drug other than L-Dopa (Pramipexole, Rotigotine, Ropinirole, Cabergoline, Pergolide, Trihexyphenidyl, Symmetrel, L-DOPS, Entacapone, Selegiline, or Zonisamde) using Wilcoxon signed rank test (Suppl. Table 1). We found no association between the nine mass numbers and the eleven drugs. Thus, the nine mass numbers were unlikely to be associated with treatment for PD.

3.5. Candidates of chemical substances for the nine mass numbers

We made PLS models again with the nine mass numbers in 18 sectionalized time windows to predict UPDRS3 (see Materials and Methods). Candidate chemical substances were estimated by inspecting retention times of the nine mass numbers in the chromatograms (Table 3). None of

Fig. 1. Representative mass chromatograms of m/e 48, 63, 67, 70, 81, 93, 96, 104 and 105 selected for SVM modeling for differentiation of PD and controls and also for SVR modeling for prediction of UPDRS3 scores. Note that m/e 70, 81, 93, and 96 had positive coefficients, whereas m/e 48, 63, 67, 104, and 105 had negative coefficients for PD in PLS analysis (Table 2).
Fig. 2. Prediction of UPDRS3 scores (A) SVR models predicting UPDRS3 scores were generated using the average peaks from 200 to 400 sec of nine mass numbers (m/e 48, 63, 67, 70, 81, 93, 96, 104, and 105). In the leave-one-out cross-validation analysis, one of 41 PD patients was excluded to generate an SVR model, and the actual and predicted UPDRS3 scores of the excluded patient are plotted on the graph. Forty-one SVR models were generated by excluding one PD patient each. The correlation coefficient was 0.710. (B) A global SVR model was generated using 41 PD patients. The correlation coefficient was 0.834. Linear regression lines are indicated by solid lines, and 95% confidence intervals are indicated by dotted lines.

Table 3. Candidate chemical substances to predict severity of PD

| Increased in severe PD patients (UPDRS3 > 20) | Increased in mild PD patients (UPDRS3 < 10) |
|---------------------------------------------|-------------------------------------------|
| Nonanal<sup>a</sup>                        | Decanal<sup>a</sup>                      |
| Heptanal<sup>a</sup>                       | Hexanal<sup>b</sup>                      |
| 2-Butenal<sup>a</sup>                      | 2-Ethylhexanol                           |
| Styrene                                     | Ethylbenzene                             |
| Ethyl acetate                               | p-Xylene                                 |
| 5-Hepten-2-one, 6-methyl                    | o-Xylene                                 |
| Higher hydrocarbons such as hexadecane,     | N,N-Dimethyl acetamide                   |
| tridecane and dodecane                      |                                           |
| Acetone                                     |                                           |

<sup>a</sup>Aldehydes

these candidate substances were known to be associated with PD pathology or PD treatment.

4. Discussion

Breath gas has been widely used in studies of human diseases [3-5], whereas the analysis of the skin gas is rare [6]. According to our personal experiences, breath gas is largely influenced by volatile substances produced or consumed by oral microbiota, whereas the skin gas is devoid of these factors. Although acquisition of the skin gas was more tedious than that of breath gas, we previously developed a method to reproducibly obtain gas samples from the skin [7-10].

We first sectionalized the original GC/MS chromatograms every 30 sec, and selected the chromatograms from 200 to 400 sec according to preliminary PLS analysis to predict the severity of motor deficits (UPDRS3 score) in PD. A similar PLS analysis showed that 50 mass numbers had high coefficients to predict the UPDRS3 scores. We individually inspected chromatographic consistency of each peak of the 50 mass numbers, and selected 9 mass numbers. We normalized for the retention time drift, and chromatograms that could not be normalized were excluded from our analysis. Scrutinizing inspection of each peak and exclusion of over-drifted chromatograms allowed us to make dependable SVM and SVR models.

To confirm that UPDRS scores can be efficiently predicted by SVR modeling using the nine mass numbers, we validated our strategy by LOOCV. The cross-validation revealed the Pearson’s correlation coefficient of 0.710 (Fig. 2A). We next generated an SVR model using the nine mass numbers of the 41 PD patients, and obtained the correlation coefficient of 0.834 (Fig. 2B).

We predicted candidate chemical substances (Table 3), which included a group of C7 and C9 aldehydes in favor of higher UPDRS3 scores, and a group of C6 and C12 aldehydes in favor of lower UPDRS3 scores. In addition to the difference in aldehydes, styrene was found in the higher UPDRS3 scores, whereas ethylbenzene, p-xylene, and o-xylene were found in the lower scores, suggesting that more reactive chemical substances like styrene were likely to be elevated in severe PD patients. We also observed that hydrocarbons (hexadecane, tridecane, and dodecane), and ethyl acetate were high in higher UPDRS3 scores. These chemical substances might be taken into the body in daily life. It is interesting to note that dimethyl acetamide, which is toxic to the liver, the pancreas, and the spleen [11], was predicted to be increased in mild PD patients.

Acetone was one of the elevated putative chemical substances with higher UPDRS3 scores (Table 3). Acetone is produced through normal metabolic processes, and is present in blood and urine. Patients with diabetes mellitus
showed mass fragments at every half second. We thus representing skin gas components. Every chemical substrate agonists for the peroxisome proliferation-activated receptor gamma (PPARγ), such as antidiabetic drug, glitazone (GTZ), are neuroprotective in animal models of PD [15] and in a retrospective cohort study of PD patients [16]. Increased acetone may indicate latent insulin resistance in our PD patients, although we did not examine the blood HbA1c levels.

Positive association of PD with environmental factors especially with rural living, well-water consumption, and the use of pesticides, herbicides, insecticides, and fungicides have been repeatedly studied but without definite conclusions [17-19]. Candidate substances shown in Table 4 may represent environmental factors that are associated with PD. Alternatively, chemical substances in the skin gas may represent abnormal skin permeability in PD. Misfolded α-synuclein fibrils, which are directly associated with dopaminergic cell death in the mid brain, accumulates in the skin [20,21]. In accordance with this abnormality, sympathetic response is compromised in PD [22]. Although no report has addressed the skin texture in PD to the best of our knowledge, these skin aberrations possibility affect the skin permeability of chemical substances in PD. Candidate chemical substances that we observed in the skin gas in PD may represent changes of the skin texture in PD.

5. Conclusions

Nine mass numbers extracted by the GC/MS analysis of skin gas collected from the hand were able to predict the severity of PD, namely the UPDRS3 score, with a correlation coefficient of 0.834, which was expected to be high enough to be applied in medical practice.

Each GC/MS chromatogram included a lot of data representing skin gas components. Every chemical substrate showed mass fragments at every half second. We thus obtained too much information on skin gas components by GC/MS from each individual, whereas we obtained a single UPDRS3 from each PD patient. In this study, we sectionalized the GC/MS chromatograms every 30 seconds, and performed PLS analysis to predict the UPDRS3 score. We also visually scrutinized each chromatogram for chromatographic consistency of each mass number. In this study, the sectionalization of chromatograms and the visual inspection of each chromatogram were critical processes to extract essential information from skin gas and to make SVM and SVR models for PD.

We suggest that further study will be required to reveal how the PD-associated chemical compounds are generated and pass through the human skin. In addition, as participants in our study were not well age- and sex-matched, feature-adjusted controls or a larger number of participants will be required to exclude possible contributions of these confounding factors. We conclude that skin gas analysis will provide substantial information to diagnose PD especially at its early stage.

Competing interests

Nothing to declare.

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Supporting information

The supplementary table showing Z scores of the nine mass numbers, UPDRS3, and L-Dopa dose of 41 PD patients are available via the WEB at http://chromsoc.jp/Journal/SI.html.

References

[1] Goetz, C. G.; Tilley, B. C.; Shaftman, S. R.; Stebbins, G. T.; Fahn, S.; Martinez-Martin, P.; Poewe, W.; Sampaio, C.; Stern, M. B.; Dodel, R.; Dubois, B.; Holloway, R.; Jankovic, J.; Kuilsevsky, J.; Lang, A. E.; Lees, A.; Leurgans, S.; LeWitt, P. A.; Nyenhuis, D.; Olanow, C. W.; Rascol, O.; Schrag, A.; Teresi, J. A.; van Hilt, J. J.; LaPelle, N.; Movement Disorder Society, U. R. T. F. Mov. Disord. 2008, 23, 2129-2170.
[2] Chang, C. C.; Lin, C. J. Acn. T. Intel. Syst. Tec. 2011, 2, Article 27.
[3] Tisch, U.; Haick, H. J. Breath Res. 2014, 8, 027103.
[4] Di Natale, C.; Paollesse, R.; Martinelli, E.; Capuano, R. Anal. Chim. Acta 2014, 824, 1-17.
[5] Wallace, M. A. G.; Pleil, J. D. Anal. Chim. Acta 2018, 1024, 18-38.
[6] Tsuda, T.; Ohkuwa, T.; Itoh, H. Findings of skin gases and their possibilities in healthcare monitoring. In Gas Biology Research in Clinical Practice, Yoshikawa, T.; Naito, Y. (Eds.) Karger: Basel, Switzerland, 2011, pp. 125-132.
[7] Naitoh, K.; Tsuda, T.; Nose, K.; Kondo, T.; Takasu, A.; Hiramotarashi, T. Instrum. Sci. Technol. 2002, 30, 267-280.
[8] Nunome, Y.; Tsuda, T.; Kitagawa, K. Anal. Sci. 2010, 26, 917-919.
[9] Yamai, K.; Funada, T.; Ohkuwa, T.; Itoh, H.; Tsuda, T. *Anal. Sci.* **2012**, *28*, 511-514.

[10] Nakanishi, R.; Ohwaki, J.; Emoto, S.; Mori, T.; Mizuno, K.; Tsuda, T.; Itoh, H.; Ohkuwa, T. *Redox Rep.* **2013**, *18*, 233-237.

[11] Kennedy, G. L. *Crit. Rev. Toxicol.* **2012**, *42*, 793-826.

[12] Hu, G.; Joussilahti, P.; Bidel, S.; Antikainen, R.; Tuomilehto, J. *Diabetes Care* **2007**, *30*, 842-847.

[13] Arvanitakis, Z.; Wilson, R. S.; Bienias, J. L.; Bennett, D. A. *Alzheimer Dis. Assoc. Disord.* **2007**, *21*, 144-149.

[14] D’Amelio, M.; Ragonese, P.; Callari, G.; Di Benedetto, N.; Palmeri, B.; Terruso, V.; Saleni, G.; Famoso, G.; Aridon, P.; Savettieri, G. *Parkinsonism Relat. Disord.* **2009**, *15*, 660-664.

[15] Carta, A. R.; Frau, L.; Pisanu, A.; Wardas, J.; Spiga, S.; Carboni, E. *Neuroscience* **2011**, *194*, 250-261.

[16] Brauer, R.; Bhaskaran, K.; Chaturvedi, N.; Dexter, D. T.; Smeeth, L.; Douglas, I. *PLoS Med.* **2015**, *12*, e1001854.

[17] Barbeau, A.; Roy, M.; Cloutier, T.; Plasse, L.; Paris, S. *Adv. Neurol.* **1987**, *45*, 299-306.

[18] Pezzoli, G.; Cereda, E. *Neurology* **2013**, *80*, 2035-2041.

[19] Breckenridge, C. B.; Berry, C.; Chang, E. T.; Stielken, R. L., Jr.; Mandel, J. S. *PLoS One* **2016**, *11*, e0151841.

[20] Gibbons, C. H.; Garcia, J.; Wang, N.; Shih, L. C.; Freeman, R. *Neurology* **2016**, *87*, 505-512.

[21] Donadio, V.; Incensi, A.; Piccinini, C.; Cortelli, P.; Giannoccaro, M. P.; Baruzzi, A.; Liguori, R. *Ann. Neurol.* **2016**, *79*, 306-316.

[22] Toru, S.; Kanouchi, T.; Yokota, T.; Yagi, Y.; Machida, A.; Kobayashi, T. *Eur. Neurol.* **2018**, *79*, 27-32.