

# Midbrain Atrophy Related to Parkinsonism in a Non-Coding Repeat Expansion Disorder: Five Cases of Spinocerebellar Ataxia Type 31 With Nigrostriatal Dopaminergic Dysfunction

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## Research

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# Abstract

## Background

Spinocerebellar ataxia type 31 (SCA31) is caused by non-coding pentanucleotide repeat expansions in the *BEAN1* gene. Clinically, SCA31 is characterized by late-adult onset, pure cerebellar ataxia. To explore the association between parkinsonism and SCA31, five patients with SCA31 with concomitant nigrostriatal dopaminergic dysfunction (NSDD) development, including three cases of L-DOPA responsive parkinsonism, were characterized.

## Methods

To assess regional brain atrophy, cross-sectional and longitudinal imaging analyses were retrospectively performed using Magnetic resonance imaging (MRI) planimetry. The midbrain-to-pons (M/P) area ratio and cerebellar area were measured on midsagittal T1-weighted MRI in five patients with NSDD (NSDD(+)), 14 patients with SCA31 without NSDD (NSDD(-)), 32 patients with Parkinson's disease (PD), and 15 patients with progressive supranuclear palsy. Longitudinal changes in the M/P area ratio were assessed by serial MRI of NSDD(+) ( $n = 5$ ) and NSDD(-) ( $n = 9$ ).

## Results

The clinical characteristics relevant to parkinsonism in NSDD(+) were indistinguishable from those of PD except for the prominence of bradykinesia/akinesia (5/5) and the absence of dysautonomia (5/5). However, a clear reduction in the midbrain area and the M/P area ratio was observed in NSDD(+) ( $p < 0.05$ ) while there was no significant difference in disease duration or in the pons area among NSDD(+), NSDD(-), and PD. A faster rate of decline in the midbrain area and the M/P area ratio was evident in NSDD(+) ( $p < 0.05$ ).

## Conclusion

The unique features of midbrain atrophy related to NSDD in our patients suggest that SCA31 belongs to the group of non-coding repeat expansion disorders causing parkinsonism.

## Introduction

Spinocerebellar ataxia type 31 (SCA31) is an inherited neurodegenerative disorder characterized by slowly progressive, late-adult onset, pure cerebellar ataxia [1, 2]. SCA31 is caused by an insertion mutation of variable-length (2.5–3.8 kb) containing (TGGAA) $n$  within the intron of the brain-expressed associated with NEDD4 1 (*BEAN1*) gene [3]. A few cases of SCA31 presenting with extracerebellar signs, including parkinsonism, postural tremor, dystonia, and spastic paraparesis, have been reported [4-6]. In Japan, genetic testing for SCA31 is usually performed in patients with pure cerebellar ataxia but yields a bias against extracerebellar manifestations of SCA31, and only a few patients with SCA31 have

undergone neuropathological assessment. Therefore, the extracerebellar manifestations in patients with SCA31 are still largely unknown.

The present study analyzed five cases of SCA31 with nigrostriatal dopaminergic dysfunction (NSDD), which is defined as the presence of at least two of the three cardinal motor symptoms of Parkinson's disease (PD), including resting tremor, rigidity, and bradykinesia/akinesia or one of the three cardinal motor symptoms plus an abnormal  $^{123}\text{I}$ -ioflupane dopamine transporter (DAT) scintigraphy finding. We aimed to describe the clinical characteristics of these cases and to explore the association between NSDD and SCA31. Cross-sectional and longitudinal imaging analyses were retrospectively conducted to assess regional brain atrophy in the midbrain and pons in the patients with NSDD and the control subjects.

## Patients And Methods

### Patients

Between April 2010 and December 2019, 20 patients with cerebellar ataxia caused by a genetic mutation responsible for SCA31 were referred to our hospital. Of these, NSDD developed in five patients. In total, 19 patients with SCA31 underwent cross-sectional imaging analysis using Magnetic resonance imaging (MRI) planimetry and were divided into the SCA31 with NSDD group (NSDD(+),  $n = 5$ ) and the SCA31 without NSDD group (NSDD(-),  $n = 14$ ). One patient was excluded due to having infarcts affecting the midline sagittal MRI assessments. Serial brain MRI examinations and longitudinal imaging analysis were performed in all five patients with NSDD(+) and in nine of the 14 patients with NSDD(-).

Between January 2019 and July 2020, 39 patients with PD who fulfilled the movement disorder society (MDS) clinical diagnostic criteria for 'clinically established PD' were referred to our hospital [7]. To exclude juvenile PD, three patients who were younger than 50 years at onset were excluded. PD is susceptible to clinical misdiagnosis, especially in its earlier stages. For this reason, four patients who had a disease duration of less than three years from onset were also excluded. In total, 32 patients with PD underwent cross-sectional imaging analysis.

Between January 2018 and July 2020, 15 patients with PSP who fulfilled the MDS clinical diagnostic criteria for 'probable PSP' were referred to our hospital [8]. All these patients underwent cross-sectional imaging analysis.

### Clinical data

Clinical data were collected from medical records. After obtaining informed consent, imaging studies of DAT scintigraphy and 3-Tesla (3-T) neuromelanin MRI were performed in four patients with NSDD. DAT scintigraphy was not performed in one patient due to the lack of consent. DAT scintigraphy and 3-T neuromelanin MRI were not performed in another patient with NSDD due to the development of lung

cancer. The 3-T neuromelanin MRI was performed in accordance with the previously described method [9].

## Gene analysis

After written informed consent was obtained, genomic DNA was extracted from peripheral-blood leukocytes and tested for SCA1, 2, 3, 6, 7, 8, 12, 17, and dentatorubral-pallidoluysian atrophy (DRPLA) using the previously described method [10]. SCA31 was diagnosed in patients showing both a single-nucleotide C→T substitution in the 5'UTR of the puratrophin-1 gene and a pentanucleotide insertion in the introns of the *TK2* and *BEAN1* genes [1-3]. To visualize the C→T substitution in the 5'UTR of the puratrophin-1 gene, the genomic DNA was amplified by PCR method (Forward primer: 5'-CAGCGCGTTTCACACTGAGA-3', Reverse primer: 5'-GGCCCTTTCTGACAGGACTGA-3'), and the PCR product was digested by EcoN1. The patients with SCA31 had the C→T substitution in the mutant allele, which disrupted one EcoNI site and produced fragments of 268 and 92 bp [11]. Analysis of the insertion mutation was performed using the following method [12]. Briefly, genomic DNA 100 ng was mixed with 10 μM primer 0.75 μl (Forward primer: 5'-ACTCCAACCTGGGATGCAGTTTCTCAAT-3', Reverse primer: 5'-CTTTAGGGACCTGATTCCTTCCTCCA-3') in a total volume of 25 μl containing 2X-PCR buffer 12.5 μl for KODFX (TOYOBO), dNTP 400 μM, H<sub>2</sub>O 5 μl, and KODFX 0.5 μl (TOYOBO). The samples were denatured at 95°C for 5 min, followed by 35 cycles at 95°C for 20 sec and at 68°C for 8 min. The PCR products were run on 1.5% agarose gel.

## MRI planimetry

All the patients underwent brain MRI with a 3-T MRI scanner (750 GE). MRI-based planimetry on midsagittal T1-weighted MRI was performed to measure the area of the midbrain, pons, and cerebellum and to calculate the midbrain-to-pons (M/P) and the cerebellum-to-pons (C/P) area ratios. The pontomesencephalic junction was defined by a line between the superior pontine notch and the inferior border of the quadrigeminal plate [13]. The pontomedullary junction was defined by a line parallel to the first line at the level of the inferior pontine notch. Image analysis was done by blinded investigators using ImageJ software (version 1.52). First, a region of interest (ROI) was located in the area of the pontine tegmentum to derive the mean individual background signal and SD. Then, the ROIs were manually outlined as described above. To delineate the boundary of the ROIs, the area in each ROI was measured by a signal intensity value higher than the mean individual background signal minus 3SD (midbrain, pons) or the mean background signal minus 8SD (cerebellum). Two neurologists acting as independent raters blinded to the diagnosis analyzed the images.

## Cross-sectional imaging analysis

The area of the midbrain, pons, and cerebellum was measured in patients with SCA31, and the area of the midbrain and pons was measured in patients with PD and PSP. The latest MRI scan was used for imaging analysis in each patient who had repetitive MRI examinations.

## Longitudinal imaging analysis

Serial MRI scans were done for all five patients with NSDD(+) and nine patients with NSDD(-). Two MRI scans were removed for further study due to severe motion artifacts. A total of 40 MRI scans (15 for the NSDD(+) group and 25 for the NSDD(-) group) were used to measure the midbrain and pons area to calculate the M/P area ratio.

## Statistical analysis

Continuous variables were checked for normality and homogeneity of variance with Shapiro–Wilk's and Levene's tests. Normally distributed data were analyzed using Student's t test or Welch test. The Mann-Whitney test was used when the variable was either ordinal or continuous, but not normally distributed. The difference between the two groups was judged to be statistically significant if the P value was 0.05 or less. Linear regression analysis was performed to determine the effect of disease duration on regional brain atrophy. Normality and homoscedasticity of the residuals were checked in the linear regression analyses. Statistical analyses were performed using IBM SPSS Statistics 20.

# Results

## Clinical characteristics of patients with SCA31 complicated with NSDD

Of the 20 genetically confirmed cases of SCA31, NSDD developed in five patients, including four presenting with rigidity and bradykinesia/akinesia and one presenting with bradykinesia/akinesia with an abnormal DAT scintigraphy finding. The clinical characteristics of cerebellar ataxia in SCA31, including slowly progressive, late-adult onset ataxia, eye movement abnormalities with saccade and horizontal gaze-evoked nystagmus, and prominent atrophy of the upper cerebellum, were observed equally in patients with and without NSDD. The clinical and imaging features of parkinsonism and the related symptoms in the five patients with NSDD (Table 1) were as follows: L-DOPA responsive parkinsonism (3/3), prominence of bradykinesia/akinesia (5/5), dysautonomia (0/5), vertical gaze limitation (1/5), pons atrophy (0/5), putamen atrophy (0/5), and abnormal DAT scintigraphy (3/3), and 3-T neuromelanin MRI (4/4) findings. NSDD developed in all the patients after the onset of cerebellar ataxia.

## Cross-sectional imaging analysis in patients with SCA31 complicated with NSDD

Table 2 summarizes the demographic features of 19 patients with SCA31 with or without NSDD, 32 patients with PD and 15 patients with PSP and the results of the cross-sectional imaging analysis. First, to estimate the influence of disease duration on the midbrain area, pons, and cerebellum, linear regression analysis was used to assess the relationship between disease duration at MRI acquisition versus M/P area ratio and the relationship between disease duration at MRI acquisition and the C/P area ratio in all 19 patients with SCA31 (Supplementary Fig. 1). As expected, there was a significant effect of disease duration on the C/P area ratio (coefficient of determination,  $R^2 = 0.305$ ,  $p = 0.014$ ). Whereas, there was no significant effect of disease duration on the M/P area ratio ( $R^2 = 0.157$ ,  $p = 0.093$ ), although there

was a weak, linear relationship between the variables. The patients were then divided into two groups according to the presence or absence of NSDD (NSDD(+): SCA31 with NSDD,  $n = 5$ , NSDD(-): SCA31 without NSDD,  $n = 14$ ). All five patients with NSDD were male; thus, NSDD(-) was subdivided into male patients without NSDD ( $n = 9$ , NSDD(-)m). An apparent reduction in the midbrain area and the M/P area ratio was observed in NSDD(+) ( $p < 0.05$ ) while there was no statically significant difference in disease duration at MRI acquisition or in the C/P area ratio among the three groups (Table 2). Furthermore, the NSDD(+) findings were compared to the findings in the 32 patients with PD and 15 patients with PSP. For statistical comparison, these patients were further divided into male patients with PD (PDm) and male patients with PSP (PSPm). There was a clear difference in the midbrain area and the M/P area ratio between the NSDD(+) and PD groups and between the NSDD(+) and PDm groups ( $p < 0.05$ ) (Table 2). In line with previous studies, the PSP group showed a severe reduction in the midbrain and pons area than that of PD group [13-16]. There was also a clear difference in the midbrain and pons area between the NSDD(+) and PSP groups and between the NSDD(+) and PSPm groups ( $p < 0.05$ ) (Table 2). These results suggest mild but significant midbrain atrophy in patients with NSDD. Figure 1 shows midbrain tegmentum atrophy in a representative case of NSDD and the result of the comparison of the M/P area ratio in these groups. Image analysis by an independent investigator confirmed these results.

### **Longitudinal imaging analysis in patients with SCA31 complicated with NSDD**

A previous, longitudinal study reported the regional rates of atrophy of 0.3%/year in the midbrain and 0.2%/year in the pons in healthy adult subjects [17]. From these data, the rate of reduction in the M/P area ratio was estimated to be 0.001/year (M/P area ratio at time 0 = 1). Thus, the M/P area ratio may be useful for minimizing the effect of age on midbrain atrophy. Longitudinal changes in the M/P area ratio were calculated using serial brain MRI obtained from patients with NSDD(+) ( $n = 5$ ) and NSDD(-) ( $n = 9$ ). The NSDD(-) group was further divided into male patients without NSDD ( $n = 6$ , NSDD(-)m). Table 3 summarizes the demographic features, average number of MRI examinations, and the mean interval between the MRI and the results of longitudinal imaging analyses in the three groups. To normalize the individual difference, the reduction ratio of the M/P area ratio was calculated as follows: the M/P area ratio derived from the first MRI examination was defined as 1, and the M/P area ratio derived from the second or later MRI was divided by the M/P area ratio derived from the first MRI in each patient. Linear regression of the reduction ratio of the M/P area ratio over time in the NSDD(+) group showed a significant effect of disease duration on the reduction ratio of the M/P area ratio ( $R^2 = 0.267$ ,  $p = 0.0488$ ) (Fig. 2A). Whereas, linear regression of the reduction ratio of the M/P area ratio over time in the NSDD(-) and NSDD(-)m groups showed no significant effect of disease duration on the reduction ratio of the M/P area ratio ( $R^2 = 0.044$ ,  $p = 0.312$  and  $R^2 = 0.031$ ,  $p = 0.471$ , respectively) (Fig. 2.B.C). The difference in the linear regression slopes (Fig. 2) between the NSDD(+) and NSDD(-) groups and between the NSDD(+) and NSDD(-)m groups was also tested (Supplementary File 1) and the result confirmed a faster rate of decline in the M/P area ratio in the NSDD(+) group. There was a clear difference in the reduction ratio of the midbrain area and the M/P area ratio in the NSDD(+) group ( $p < 0.05$ ) while no significant difference in the reduction ratio of the pons area or in the mean interval between the MRI was observed among the

three groups (Table 3). Thus, a faster rate of decline in the midbrain area as well as the M/P area ratio was evident in the NSDD(+) group. These results suggest a significant progression of midbrain atrophy in patient with NSDD.

## Discussion

The M/P area ratio as measured on the midline sagittal images is reportedly a simple and reliable metric for distinguishing PSP from PD and multiple system atrophy (MSA) [13-16]. A statistically significant decrease in the midbrain area and the M/P area ratio was found in patients with SCA31 complicated with NSDD (Table 2, Fig. 1). Compared to the PSP findings, the midbrain atrophy was mild and no pontine atrophy was evident. Further observation of the progression of the midbrain atrophy in these patients suggested that a relationship exists between midbrain atrophy and NSDD pathogenicity. Midbrain atrophy was also reported in patients with vascular parkinsonism [18]. In the present study, all five patients with NSDD had no medical history of stroke or diffuse subcortical white matter ischemia. The clinical characteristics relevant to parkinsonism in the patients with NSDD, together with the topographical pattern of atrophy, were inconsistent with those of PD, PSP or MSA. Furthermore, genetic testing excluded the other types of SCA, including SCA1, 2, 3, 6, 7, 8, 12, 17, and DRPLA. Thus, the findings of the present study raised the possibility of an association between NSDD and the pathomechanisms underlying SCA31 despite the fact that to date, only a single-case report describing an association between SCA31 and parkinsonism has been published [4]. Importantly, compared to a typical patient with PD, the patients in the present study showed mild rigidity due to the hypotonia of cerebellar injury before parkinsonism onset, prominence of bradykinesia/akinesia, and reduced spontaneous activity, including speech and voluntary movements. These features may lead to an underestimation of parkinsonism in patients with SCA31.

SCA31 is a form of non-coding repeat expansion disorders that can be pathologically characterized by RNA foci formation and repeat-associated non-AUG translation [19-20]. The underlying mechanism of non-coding repeat sequence mediated neurotoxicity is still uncertain. However, a previous study reported that above a pathological threshold repeat number, base pairing interactions drive liquid phase separation of RNA into membraneless gels as RNA foci [21]. The gelation of intracellular compartments may result in neurotoxicity by sequestering RNA binding proteins (RBPs) and inhibiting their normal function [21, 22]. A recent study found that ALS-linked RBP, TDP-43, and FUS, bound to and induced structural alteration of SCA31-associated UGGAA repeat expansion, with a formation of RNA foci [23]. Interestingly, a mutation in the *TARDBP* gene encoding TDP-43 was identified as a cause of familial PD, presumably due to loss of TDP-43 function [24]. Recently, the authors of the present study experienced a case of essential tremor with no other neurological findings, including cerebellar ataxia, in which the results of a genetic test revealed an insertion mutation responsible for the SCA31 phenotype. Another study found that a loss-of-function mutation in the *FUS* gene caused essential tremor [25]. Thus, loss of function of RBPs binding specifically to UGGAA repeats within RNA foci can potentially explain the extracerebellar manifestations of SCA31.

The present study has several limitations. Because the study was retrospective and enrolled a small number of subjects, estimating the actual incidence of NSDD in SCA31 and the gender difference in relation to the risk of developing NSDD was difficult. All five patients with NSDD were male, and SCA31 was characterized by late-adult onset. Therefore, gender and age effects on regional brain atrophy were carefully considered. To minimize these effects, the male patients with NSDD(-), PD or PSP were compared statistically and longitudinal changes in the M/P area ratio were assessed. Indeed, the M/P area ratio minimized the effect of age on regional brain atrophy (Fig. 2.B.C). A growing number of diseases caused by non-coding repeat expansions are often associated with parkinsonism, including fragile X-associated tremor/ataxia syndrome (FXTAS) and neuronal intranuclear inclusion disease [26, 27]. Midbrain atrophy-related parkinsonism was reported in patients with FXTAS although its association with this disease remains elusive [28-30]. Further studies are required to clarify the association between NSDD and SCA31. This in turn will be helpful in understanding the pathomechanisms underlying SCA31 and parkinsonism associated with non-coding repeat expansion disorders.

## **Conclusion**

The present study revealed unique features of NSDD and shed light on its relationship to midbrain atrophy in patients with SCA 31, suggesting that SCA31 belongs to the group of non-coding repeat expansion disorders causing parkinsonism

## **Declarations**

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### **Ethics declarations**

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### **Conflicts of interest/Competing interests**

The authors declare no conflicts of interest associated with this manuscript

### **Ethics approval**

The local Ethical Committee of Tokyo Metropolitan Neurological Hospital supervised and approved all procedures (TS-R02-016, TS-R02-033), including utilization of datasets of the control subjects (PD and PSP), in accordance with the Declaration of Helsinki (amended version 2013). All patients with SCA31

provided written informed consent for the genetic analysis, the use of their clinical data for research purposes, and for publication. Under Japan law, no additional ethical approval is required for retrospective database studies.

### **Availability of data and material**

Data is available on the reasonable request to the corresponding author.

### **Code availability**

Not applicable.

### **Author contributions**

RN and KS contributed to the study concept and design; acquisition, analysis and interpretation of data; imaging analysis; drafting the manuscript; critical revision of the manuscript for important intellectual content. AM undertook imaging analysis; interpretation of data and critical revision of the manuscript for important intellectual content. TK and AK undertook acquisition, analysis and interpretation of data; critical revision of the manuscript for important intellectual content. ST and KT interpretation of data; critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript for publication.

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## Tables

**Table 1. Clinical characteristics of five patients with SCA31 complicated with NSDD**

Patient	1	2	3	4	5
Sex	M	M	M	M	M
Age at onset	52	57	64	73	72
Age at NSDD onset	59	60	84	74	83
Age at MRI	63	61	85	77	83
Family history	+	+	+	-	+
L-DOPA response	+	+	+	NE	NE
<b>Symptoms</b>					
Cerebellar ataxia	+	+	+	+	+
Rigidity	+	+	+	-	+
Bradykinesia/akinesia	+	+	+	+	+
Tremor	+	-	-	-	+
Vertical gaze palsy	-	-	+	-	-
Increased DTR	+	+	+	-	+
Dysautonomia	-	-	-	-	-
<b>Brain MRI findings</b>					
Cerebellar atrophy	+	+	+	+	+
MCP atrophy	-	-	-	-	-
Putamen atrophy	-	-	-	-	-
Hot cross bun sign	-	-	-	-	-
<b>Neuromelanin MRI</b>					
SN melanin signal	↓	↓	↓	↓	NE
LC melanin signal	↓	↓	↓	→	NE
<b>RI findings</b>					
DAT scintigraphy	0.44/0.04	3.20/3.39	NE	3.08/2.71	NE
SBR (Right/Left)					

## Abbreviations

DAT, <sup>123</sup>I-ioflupane dopamine transporter; DTR, deep tendon reflex; LC, locus coeruleus; MCP, middle cerebellar peduncle; NE, not examined; NSDD, nigrostriatal dopaminergic dysfunction; SCA31,

spinocerebellar ataxia type 31; SBR, specific binding ratio; SN, substantia nigra.

**Table 2. Demographic features and results of cross-sectional imaging analysis using MRI planimetry**

	NSDD(+)	NSDD(-)	NSDD(-)m	PD	PDm	PSP	PSPm
<b>Number</b>	5	14	8	32	17	15	8
<b>M : F ratio</b>	5 : 0	8 : 6	8 : 0	17 : 15	17 : 0	8 : 7	8 : 0
<b>Age at disease onset</b>	63.6 ± 8.2 (52-73)	57.6 ± 7.8 (40-70) <i>p</i> = 0.191	54.6 ± 8.0 (40-65) <i>p</i> = 0.101	66.0 ± 6.2 (53-79) <i>p</i> = 0.462	67.0 ± 5.2 (55-75) <i>p</i> = 0.302	72.1 ± 8.2 (60-87) <i>p</i> = 0.074	67.9 ± 7.5 (60-85) <i>p</i> = 0.396
<b>Age at MRI</b>	74.0 ± 11.3 (61-85)	68.4 ± 13.5 (44-87) <i>p</i> = 0.415	65.3 ± 14.9 (44-87) <i>p</i> = 0.285	74.4 ± 6.0 (61-83) <i>p</i> = 0.936	75.7 ± 5.3 (66-83) <i>p</i> = 0.758	76.7 ± 7.7 (62-89) <i>p</i> = 0.546	72.8 ± 7.8 (62-88) <i>p</i> = 0.817
<b>Disease duration at MRI (year)</b>	10.4 ± 6.8 (4-21)	10.7 ± 8.2 (2-30) <i>p</i> = 0.889	10.6 ± 9.5 (2-30) <i>p</i> = 0.711	8.4 ± 3.8 (3-18) <i>p</i> = 0.562	8.7 ± 3.5 (3-17) <i>p</i> = 0.664	4.7 ± 3.1 (1-11) <i>p</i> = 0.028*	4.9 ± 3.3 (1-10) <i>p</i> = 0.047*
<b>Midbrain area (mm<sup>2</sup>)</b>	88.8 ± 15.2 (71.9-105.2)	122.2 ± 24.7 (82.7-172.6) <i>p</i> = 0.012*	122.0 ± 30.6 (82.7-172.6) <i>p</i> = 0.048*	113.3 ± 17.1 (72.1-147.3) <i>p</i> = 0.005*	114.1 ± 15.6 (88.7-141.7) <i>p</i> = 0.005*	67.6 ± 14.8 (40.9-96.3) <i>p</i> = 0.013*	62.8 ± 10.9 (40.9-73.8) <i>p</i> = 0.004*
<b>Pons area (mm<sup>2</sup>)</b>	512.5 ± 46.0 (455.2-566.9)	500.9 ± 43.8 (408.0-572.9) <i>p</i> = 0.620	514.7 ± 41.8 (459.8-572.9) <i>p</i> = 0.931	508.5 ± 63.1 (370.5-676.4) <i>p</i> = 0.890	535.6 ± 66.7 (418.0-676.4) <i>p</i> = 0.481	446.2 ± 46.8 (354.3-510.4) <i>p</i> = 0.013*	458.0 ± 41.6 (381.7-510.4) <i>p</i> = 0.049*
<b>Cerebellum area (mm<sup>2</sup>)</b>	622.7 ± 96.8 (501.8-732.0)	628.8 ± 102.2 (449.0-812.8) <i>p</i> = 0.909	649.9 ± 92.6 (510.3-812.8) <i>p</i> = 0.623				
<b>M/P area ratio</b>	0.173 ± 0.023	0.244 ± 0.047	0.235 ± 0.049	0.224 ± 0.031	0.215 ± 0.027	0.151 ± 0.026	0.137 ± 0.017

	(0.148- 0.202)	(0.180- 0.333)	(0.180- 0.333)	(0.146- 0.296)	(0.146- 0.251)	(0.107- 0.210)	(0.107- 0.157)
		<i>p</i> = 0.005*	<i>p</i> = 0.022*	<i>p</i> = 0.001*	<i>p</i> = 0.006*	<i>p</i> = 0.111	<i>p</i> = 0.007*
<b>C/P area ratio</b>	1.214 ± 0.142	1.255 ± 0.176	1.278 ± 0.174				
	(1.034- 1.395)	(0.927- 1.591)	(1.119- 1.613)				
		<i>p</i> = 0.639	<i>p</i> = 0.502				

Data are expressed as the mean ± SD (range).

\* Significant difference between NSDD(+) and X, X = NSDD(-), NSDD(-)m, PD, PDm, PSP, PSPm.

### Abbreviations

C/P, cerebellum-to-pons; M/P, midbrain-to-pons; NSDD, nigrostriatal dopaminergic dysfunction; NSDD(+), SCA31 with NSDD; NSDD(-), SCA31 without NSDD; NSDD(-)m, male patients with SCA31 without NSDD; PD, Parkinson's disease; PDm, male patients with PD; PSP, progressive supranuclear palsy; PSPm, male patients with PSP; SCA31, spinocerebellar ataxia type 31.

**Table 3. Demographic features and results of longitudinal imaging analysis using MRI planimetry**

	NSDD(+)	NSDD(-)	NSDD(-)m
<b>Number</b>	5	9	6
<b>M : F ratio</b>	5 : 0	6 : 3	6 : 0
<b>MRI number</b>	3.0 ± 0.7 (2-4)	2.8 ± 1.0 (2-5) <i>p</i> = 0.422	3.2 ± 1.0 (2-5) <i>p</i> = 0.916
<b>Age at MRI</b>	69.2 ± 9.5 (56-86)	69.1 ± 10.3 (53-87) <i>p</i> = 0.970	69.3 ± 10.4 (53-87) <i>p</i> = 0.976
<b>Disease duration at MRI (year)</b>	6.5 ± 5.4 (1-21)	10.0 ± 8.5 (1-30) <i>p</i> = 0.175	11.4 ± 9.1 (1-30) <i>p</i> = 0.087
<b>MRI interval period (year)</b>	3.2 ± 3.7 (0.0-10.8)	3.1 ± 4.0 (0.0-13.0) <i>p</i> = 0.842	3.6 ± 4.3 (0.0-13.0) <i>p</i> = 0.819
<b>Midbrain area (mm<sup>2</sup>)</b>	98.1±14.5(71.9-120.2)	114.1±20.7 (82.7-149.8) <i>p</i> = 0.012*	110.5±20.3 (82.7-149.5) <i>p</i> = 0.047*
<b>Pons area (mm<sup>2</sup>)</b>	526.0 ± 42.3 (455.2-604.9)	506.7 ± 34.0 (454.0-575.5), <i>p</i> = 0.121	507.6 ± 38.6 (454.0-575.5) <i>p</i> = 0.197
<b>M/P area ratio</b>	0.187 ± 0.026 (0.148-0.238)	0.224 ± 0.034 (0.176-0.306) <i>p</i> = 0.001*	0.216 ± 0.026 (0.176-0.260) <i>p</i> = 0.003*
<b>Reduction ratio of midbrain area</b>	0.898 ± 0.074 (0.773-0.995), n = 10	0.967 ± 0.046 (0.886-1.040), n = 16 <i>p</i> = 0.007*	0.968 ± 0.043 (0.893-1.040), n = 13 <i>p</i> = 0.009*
<b>Reduction ratio of pons area</b>	0.975 ± 0.062 (0.847-1.086), n = 10	0.979 ± 0.026 (0.943-1.033), n = 16 <i>p</i> = 0.859	0.976 ± 0.024 (0.943-1.017), n = 13 <i>p</i> = 0.985
<b>Reduction ratio of M/P area ratio</b>	0.922 ± 0.078 (0.820-1.085), n = 10	0.988 ± 0.049 (0.920-1.080), n = 16 <i>p</i> = 0.014*	0.993 ± 0.051 (0.928-1.080), n = 13 <i>p</i> = 0.016*

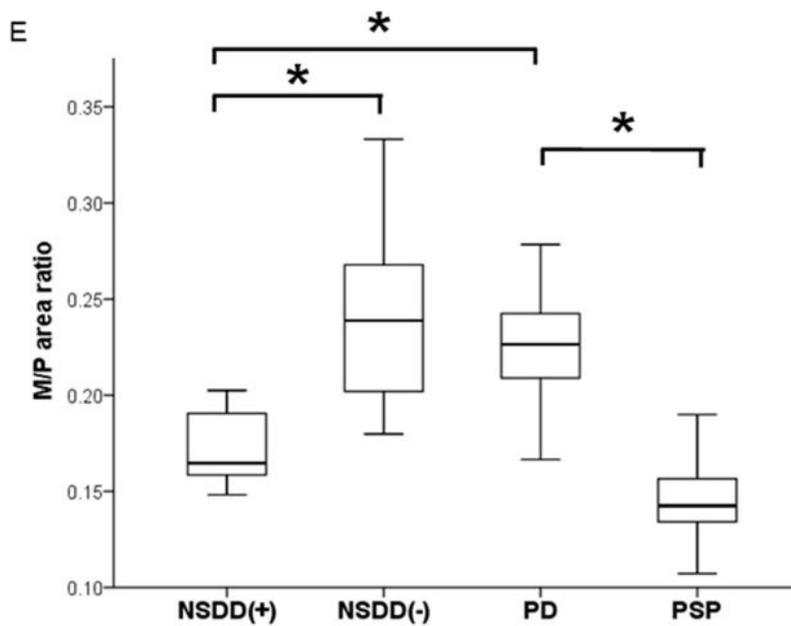
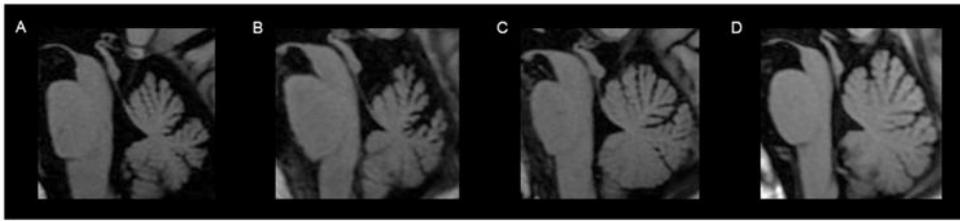
Data are expressed as the mean ± SD (range).

\* Significant difference between NSDD(+) and NSDD(-) and between NSDD(+) and NSDD(-)m.

A total of 40 MRI scans (15 for NSDD(+), 25 for NSDD(-), and 19 for NSDD(-)m) were used to measure the midbrain and pons area to calculate the M/P area ratio.

**Abbreviations:** M/P, midbrain-to-pons; NSDD, nigrostriatal dopaminergic dysfunction; NSDD(+), SCA31 with NSDD; NSDD(-), SCA31 without NSDD; NSDD(-)m, male patients with SCA31 without NSDD; SCA31, spinocerebellar ataxia type 31.

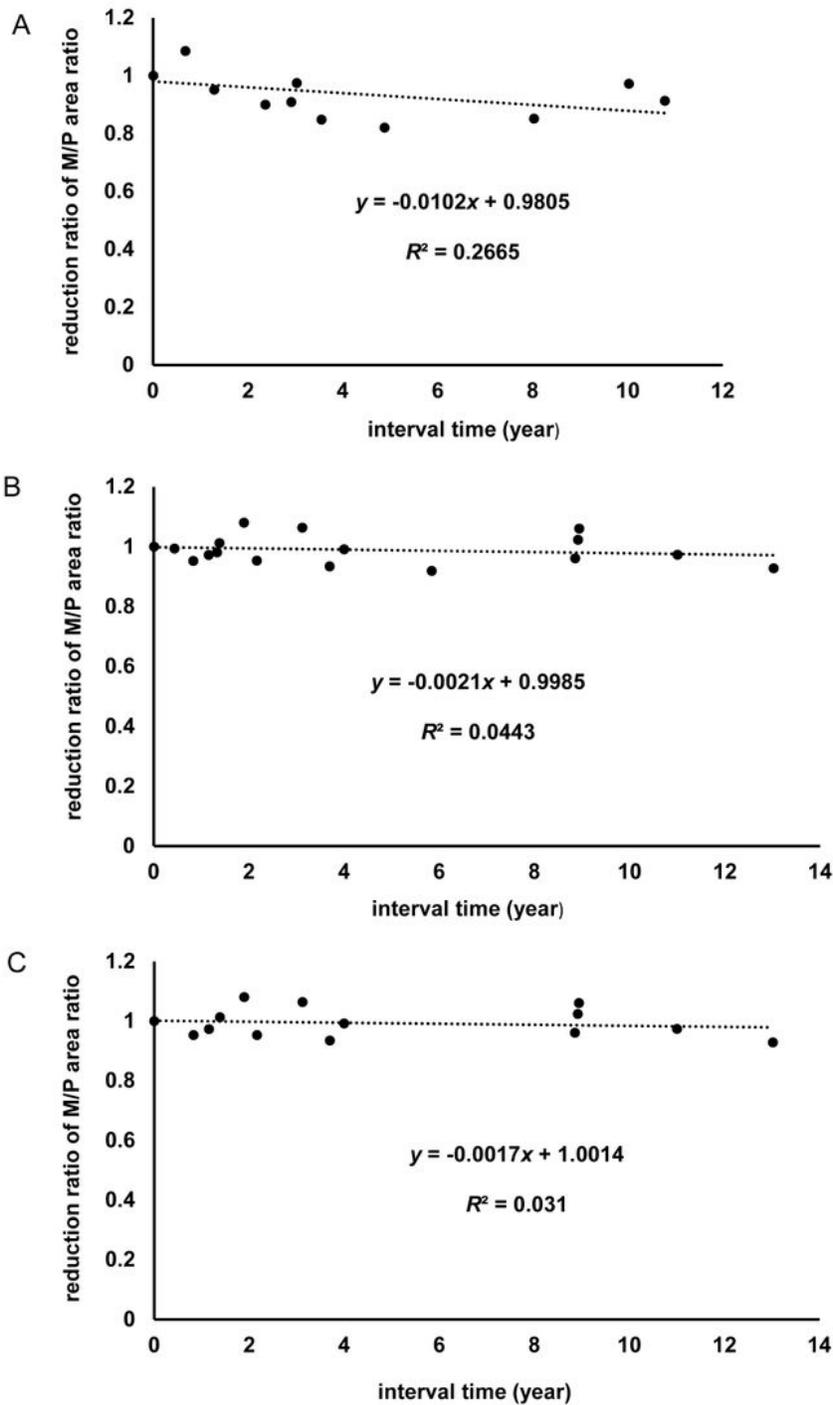
## Figures



**Figure 1**

Midbrain atrophy in a representative case of NSDD and the results of M/P area ratio Midsagittal T1-weighted MRI of a representative case from the NSDD(+) (A), NSDD(-) (B), PD (C), and PSP (D). (E) Cross-sectional imaging analysis of the M/P area ratio showing a clear difference between the NSDD(+) and NSDD(-) groups ( $p = 0.005$ ), and between the NSDD(+) and PD groups ( $p = 0.001$ ). In line with previous studies, the M/P area ratio showed a clear difference between the PD and PSP groups ( $p < 0.001$ ).

\*Significant difference ( $p < 0.05$ ). M/P, midbrain-to-pons; NSDD, nigrostriatal dopaminergic dysfunction; NSDD(+), SCA31 with NSDD; NSDD(-), SCA31 without NSDD; PD, Parkinson's disease; PSP, progressive supranuclear palsy; SCA31, spinocerebellar ataxia type 31.



**Figure 2**

Linear regression analyses of the reduction ratio of the M/P area ratio over time. Longitudinal changes in the M/P area ratio were calculated by serial brain MRI obtained from the NSDD(+) ( $n = 5$ ) (A) and NSDD(-)

(n = 9) (B) groups. Male patients in the NSDD(-) group were placed into the male patients with NSDD(-) group (n = 6, NSDD(-)m) (C). x represents the time interval (year) from the day of the first MRI examination to the second or later MRI examination in each patient. The dependent variable y represents the reduction ratio of the M/P area ratio. The M/P area ratio derived from the first MRI examination was defined as 1, and the M/P area ratio derived from the second or later MRI was divided by the M/P area ratio derived from the first MRI in each patient. M/P, midbrain-to-pons; NSDD, nigrostriatal dopaminergic dysfunction; NSDD(+), SCA31 with NSDD; NSDD(-), SCA31 without NSDD; NSDD(-)m, male patients with SCA31 without NSDD; SCA31, spinocerebellar ataxia type 31.

## Supplementary Files

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