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ORIGINAL PAPER

PSP-Phenotype in SCA8: Case Report and Systemic Review

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Abstract

Spinocerebellar ataxia type 8 (SCA8) is a rare autosomal dominant neurodegenerative disease caused by expanded CTA/CTG repeats in the *ATXN8OS* gene. Many patients had pure cerebellar ataxia, while some had parkinsonism, both without causal explanation. We analyzed the *ATXN8OS* gene in 150 Japanese patients with ataxia and 76 patients with Parkinson's disease or related disorders. We systematically reassessed 123 patients with SCA8, both our patients and those reported in other studies. Two patients with progressive supranuclear palsy (PSP) had mutations in the *ATXN8OS* gene. Systematic analyses revealed that patients with parkinsonism had significantly shorter CTA/CTG repeat expansions and older age at onset than those with predominant ataxia. We show the imaging results of patients with and without parkinsonism. We also found a significant inverse relationship between repeat sizes and age at onset in all patients, which has not been detected previously. Our results may be useful to genetic counseling, improve understanding of the pathomechanism, and extend the clinical phenotype of SCA8.

Keywords Spinocerebellar ataxia type 8 · CTA/CTG repeats · Triplet repeat · Intermediate expansion · Parkinsonism

Introduction

Spinocerebellar ataxia type 8 (SCA8) is a rare autosomal dominant neurodegenerative disease mainly associated with pure cerebellar ataxia [1, 2] and is caused by expanded CTA/CTG repeats in an untranslated region of the *ATXN8OS (ATAXIN 8 OPPOSITE STRAND)* gene [1, 2]. Recent reports have stated that expansions of repeats in the *ATXN8OS* gene have been found in several patients with Parkinson's disease, corticobasal degeneration, or atypical parkinsonism, some of whom had intermediate expansions [3–5]. Such phenotypic variations have been reported without causal explanations.

Progressive supranuclear palsy (PSP) is a relatively rare Parkinson's disease-related disorder; its pathogenic factor has not been completely established. Its prevalence in Japan is approximately 6/100,000, which is rarer than that of Parkinson's disease (150/100,000). A small percentage of patients with PSP present with cerebellar ataxia and/or cerebellar atrophy on MRI; these features are also found in SCA8 [6]. Despite having common features, the relationship between ataxia and SCA8 has not been described.

The pathogenicity of the expanded allele in the *ATXN8OS* gene has been proven using a transgenic mouse model [7]; however, its precise pathomechanism remains unestablished. Possible pathomechanisms include an abnormal RNA metabolism associated with RNA foci, poly-alanine or other polypeptides created by repeat-associated non-ATG translation, and polyglutamine protein translated from CAG repeats of the *ATXN8* gene. Normal sizes of CTA/CTG repeats range from 15 to 50, while 80 or longer repeats are pathogenic. In several reports, expansions of > 50 CTA/CTG repeats, including intermediate expansions, caused ataxia at some point in the life, without clinical details [8, 9]. Although repeat sizes in many triplet repeat diseases are inversely correlated with age

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at onset, such a correlation has not been established in patients with SCA8 [8, 9]. However, most of those studies included less than 10 patients, which would suggest that their cohorts had low statistical power. Alternatively, some of those studies included heterogeneous groups of patients with different pathomechanisms.

In this report, we analyzed the *ATXN8OS* gene in 226 patients with ataxia or parkinsonism, and reassessed 123 patients, both our patients with SCA8 and those reported in the other studies for analyzing repeat sizes, age at onset, and phenotypes. We also summarized the results of brain functional imaging in the present and reported patients with SCA8, including our patient with PSP. We also show a series of MRIs of a patient with PSP.

Materials and Methods

Genetic Testing

We analyzed genes for SCA1, 2, 3, 6, 7, 8, 12, 17, 31, and 36, and dentatorubral-pallidoluysian (DRPLA) in 150 Japanese patients with familial or sporadic ataxia, according to our previous method [10]. All exons in the *COQ2* gene, causative for multiple system atrophy, and those in the *APTX* gene, causative for the most common autosomal recessive ataxia in Japan, were sequenced in mutation-positive patients, as reported [10]. We also analyzed the *ATXN8OS* gene in 76 Japanese patients who had a clinical diagnosis of Parkinson's disease or related disorders (52 with Parkinson's disease, 6 with multiple system atrophy parkinsonism-type, 13 with PSP, and 5 with corticobasal degeneration). Additionally, we analyzed *MAPT* mutations; the *MAPT* H1 haplotype; and genes for SCA1, 2, 3, 6, 7, 8, 12, 17, 31, and 36, and dentatorubral-pallidoluysian (DRPLA) in mutation-positive patients with parkinsonism, according to the methods reported previously [11, 12]. We also analyzed the *PRKCG* gene for SCA14, a relatively frequent causative for autosomal dominant ataxia in Japan, in mutation-positive patients with ataxia according to our previously reported method [13].

Clinical Information on Patients Reported to Have SCA8

Based on the Preferred Reporting Items for Systemic Reviews and Meta-analyses (PRISMA) statement, we identified 143 patients with symptomatic SCA8, with available clinical information, from 23 studies published between 2000 and 2017 (please see the supplemental document for references). We also included data from five of our patients with SCA8. After the removal of duplicates, data from 132 patients were included. We further excluded data for patients who had an undetermined repeat size or had other causative factors present, such as mutations in other genes, vitamin E deficiency, or autoantibodies associated with cerebellar ataxia. Data for very unusual presentations, such as Huntington's disease, amyotrophic lateral sclerosis, or myoclonic epilepsy, were also excluded. We included the data of a patient with multiple system atrophy but without parkinsonism in the data for predominant ataxia [14]. We ultimately analyzed data for 123 patients, including 34 Japanese patients. The summary table of the age at onset and repeat size will be provided upon request. We statistically analyzed phenotype differences, such as predominant ataxia ($n = 106$) and parkinsonism ($n = 17$). For comparison, we further divided the patients with parkinsonism into two groups: patients who showed consistent excellent levodopa-responsiveness (levodopa-responsive parkinsonism, $n = 10$) and others (atypical parkinsonism, $n = 7$). Unfortunately, the dosage of levodopa and the duration of responsiveness were not clearly specified in most studies. We also analyzed the age at onset and repeat sizes in all patients with SCA8.

We analyzed repeat sizes of 93 unaffected individuals, as reported in 13 studies [1, 8, 15–25]. Age at examination was available for only 42 individuals; this information is summarized in a figure and compared with the oldest ages at the onset of patients with similar repeat sizes (within 10 repeat sizes).

Functional Imaging Results

We summarized the results of functional imaging, such as cerebral perfusion SPECT, ^{123}I-meta-iodobenzylguanidine (MIBG) scintigraphy, and dopamine transporter imaging in the present and reported patients with SCA8, together with clinical and genetic results. Decreased MIBG uptake supports a diagnosis of Parkinson's disease, but not of other types of parkinsonism.

Results

Results of Genetic Testing of Our Patients

Genetic testing revealed that three of the 150 patients with predominant ataxia and two of the 76 patients with parkinsonism (2 of the 13 patients with PSP) had expansions in the *ATXN8OS* gene (Fig. 1). Of the three ataxic patients, one patient had an intermediate expansion of CTA/CTG repeats: 74 repeats ($CTA_{16}CTG_6CTA_1CTG_{51}$). Interrupted CTA in CTG repeats has been already reported with unknown significance [9]. Our other patients had 151 repeats (CTA_7CTG_{144}) and 165 repeats (CTA_7CTG_{158}) respectively. Two patients with PSP in our cohort had 131 repeats ($CTA_{10}CTG_{121}$) and 93 repeats ($CTA_{16}CTG_1CTA_1CTG_{75}$). Our patients had no additional mutations in other genes tested. The two patients with PSP showed *MAPT* H1/H1 diplotype (a risk diplotype

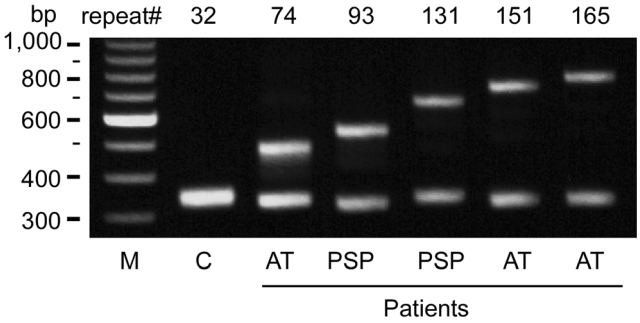

Fig. 1 The result of agarose gel electrophoretic analysis for the *ATXN8OS* gene in our patient. Five patients had expansions of CTA/CTG repeat as indicated, while control (C) had a normal repeat size. AT ataxia, PSP progressive supranuclear palsy, M 100 base pair (bp) marker

for parkinsonism mainly in Caucasians); however, the H1 haplotype was exclusively found in Japanese population without the H2 haplotype (a non-risk haplotype) [12].

Phenotype Differences and Repeat Sizes for Systemic Review

We found that patients with parkinsonism had significantly shorter repeat sizes than the other patients who predominantly had ataxia (Mann-Whitney *U* test, $p < 0.01$, Fig. 2a). Patients with parkinsonism had older age at onset than those with predominant ataxia (Mann-Whitney *U* test, $p < 0.01$, Fig. 2b). Patients with levodopa-responsive parkinsonism had significantly shorter repeats as compared to patients with atypical parkinsonism (Mann-Whitney *U* test, $p < 0.05$, Fig. 2c).

Correlation Between Repeat Sizes and Age at Onset

We collected clinical and genetic information on 123 patients with SCA8. Twelve patients (11%) were homozygotes or compound heterozygotes for CTA/CTG repeats. We initially included larger repeat sizes for the following analyses. Linear regression analyses for all 123 patients revealed that CTA/CTG repeat sizes were significantly inversely correlated with age at onset ($p < 0.01$, Fig. 3a). Six of the eight patients with ≥ 200 repeats had older age at onset than expected. A closer relationship was observed, when repeat sizes were limited to less than 200 ($p < 0.0001$, Fig. 3b). In contrast, no relationship was found between repeat sizes ≥ 200 and age at onset (not shown). We compared the phenotypes of patients having ≥ 200 repeats with the phenotypes of those having < 200 repeats; however, no significant difference was observed (not shown, Fisher's exact test, $p = 1.0$). When mean repeat sizes were included instead of larger repeat sizes in patients with compound heterozygotes, a closer relationship was observed ($p < 0.01$, Fig. 3c). Linear regression analyses for 34 Japanese patients revealed that CTA/CTG repeat sizes, including larger repeat sizes for homozygotes and compound heterozygotes, were significantly inversely correlated with age at onset ($p < 0.05$, Fig. 3d).

Results of Analyses for Homozygotes or Compound Heterozygotes

When homozygotes or compound heterozygotes were excluded from the analysis, correlation was present in heterozygotes (not shown). Comparison of age at onset between 15 patients with homozygotes and compound heterozygotes (larger repeat 111–197; mean repeat, 138) and 50 patients with heterozygotes with repeat sizes of the same range (mean repeat, 139) unexpectedly revealed a significant older age at onset in homozygotes/compound heterozygotes than in heterozygotes (43 vs. 32 years, respectively, not shown; Mann-Whitney *U* test, $p = 0.024$). To avoid potential selection bias due to the collection of data pertaining to different racial and ethnic groups, we only used data from studies that simultaneously included heterozygotes and homozygotes/compound heterozygotes with equivalent repeat sizes (26 heterozygotes and 10 homozygotes/compound heterozygotes). No significant between-group difference was observed with respect to the age at onset (Mann-Whitney *U* test, $p = 0.13$).

Clinical Presentation and Imaging Results of Patients with PSP and a Patient with an Intermediate Repeat

Two Patients with PSP

A 77-year-old man with 131 repeats walked with short steps since the age of 76 years. Concurrently, he had hesitation in his gait, difficulty in standing, and mild constipation. He had no family history or previous history of neurological diseases. His symptoms gradually worsened. He then visited our clinic at the age of 77 years. Neurologic examinations revealed neck dystonia, postural instability, and mild cogwheel rigidity in the bilateral upper limbs but normal eye movements. Pronation and supination of the upper limbs and finger tap were bilaterally affected with the left predominant involvement. He walked with a tendency to bend forward. Muscle weakness or abnormal deep tendon reflexes were not observed. Autonomic dysfunction was not apparent, except for the presence of mild constipation. His parkinsonism was unresponsive to oral levodopa (300 mg/day). Brain MRI showed modest cerebellar atrophy with mild atrophy in the frontal lobes and a relatively unaffected brainstem (Fig. 4a–d). IMP-SPECT revealed decreased perfusion in the cerebellum but preserved perfusion in the bilateral frontal lobes (Fig. 4e), although cerebellar ataxia was not apparent. MIBG scintigraphy was normal, with a mild increase in the washout ratio (51.6%, not shown). Dopamine transporter imaging using ^{123}I-ioflupane revealed a mildly decreased uptake in the bilateral putamen, with right predominance. The specific binding ratio (SBR) was 4.60 on the right side and 5.11 on the left side (cut-off

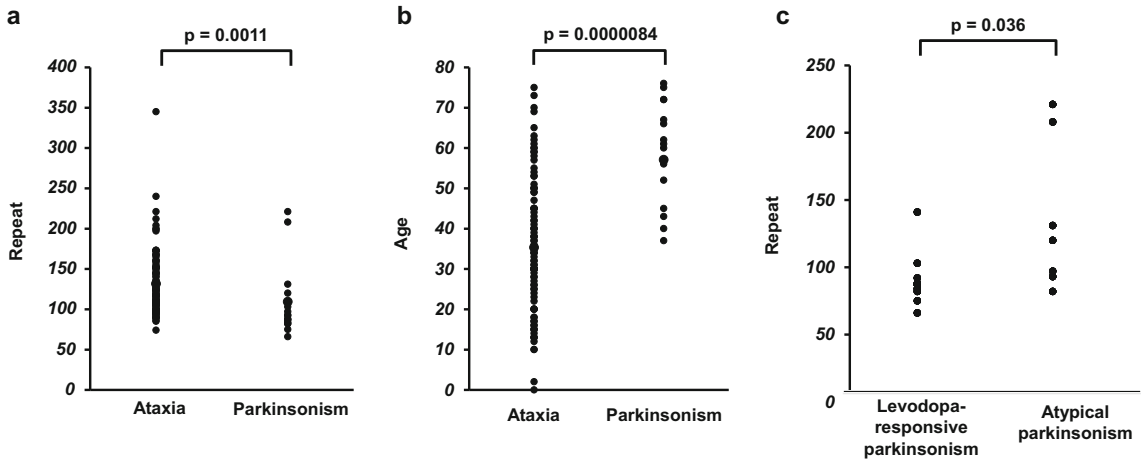

Fig. 2 Significant relationship of phenotypes with repeat sizes and age at onset. **a** Patients with parkinsonism had significantly shorter repeats than those with predominant ataxia (Mann-Whitney *U* test, $p < 0.01$). **b** Patients with parkinsonism had significantly older age at onset than those with predominant ataxia (Mann-Whitney *U* test, $p < 0.01$). **c** Patients with levodopa-responsive parkinsonism had shorter repeats than those with atypical parkinsonism (Mann-Whitney *U* test, $p < 0.05$)

point, 6.50) in our institute (Fig. 4f). His mini-mental state examination (MMSE) score was 25 (normal, > 24). Subsequently, he sustained a fall and was admitted to another hospital. Eventually, he was lost to follow-up owing to his relocation to another prefecture. According to the recent clinical diagnostic criteria, he was diagnosed as a case of suspected PSP with predominant parkinsonism [26].

A 79-year-old man with 93 repeats presented with an unsteady gait at the age of 67 years at a local hospital. Neurologic examinations revealed indications of parkinsonism, such as a masked face, dysarthria, bradykinesia, and antepulsion; he then received levodopa and initially showed a mild response. He had no family history or previous history of neurological diseases. An MRI at the age of 69 years

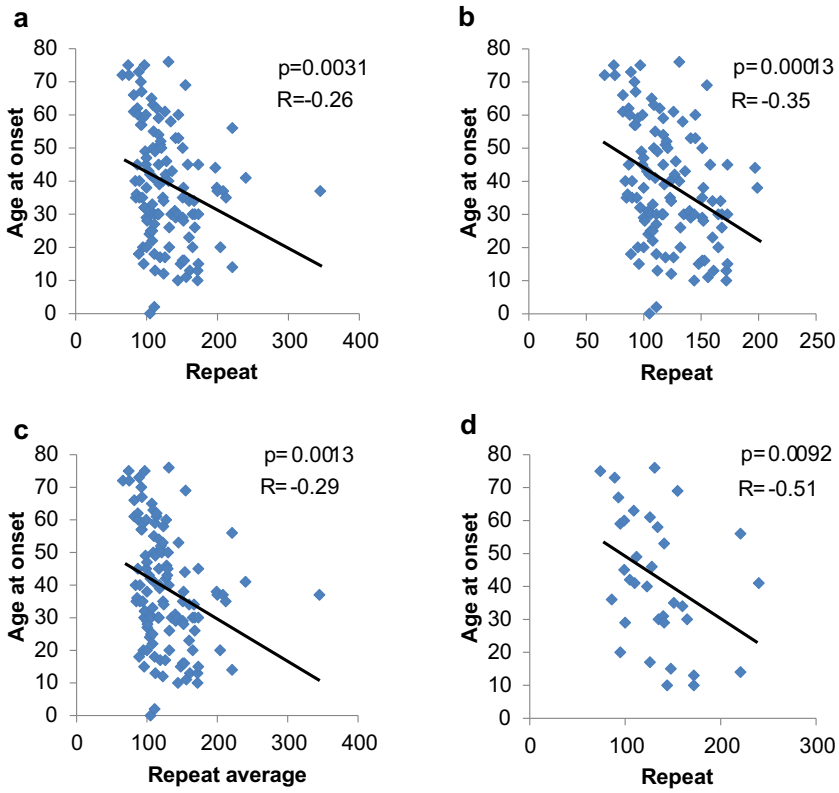

Fig. 3 Results of linear regression studies. **a** Repeat sizes were significantly correlated with age at onset in 123 patients with SCA8. **b** Repeat sizes < 200 were more closely associated with age at onset ($N = 116$). **c** The use of mean repeat sizes for compound heterozygotes together with expanded repeat sizes in heterozygotes showed a closer relationship ($N = 123$). **d** Repeat sizes were significantly correlated with age at onset in 34 Japanese patients with SCA8

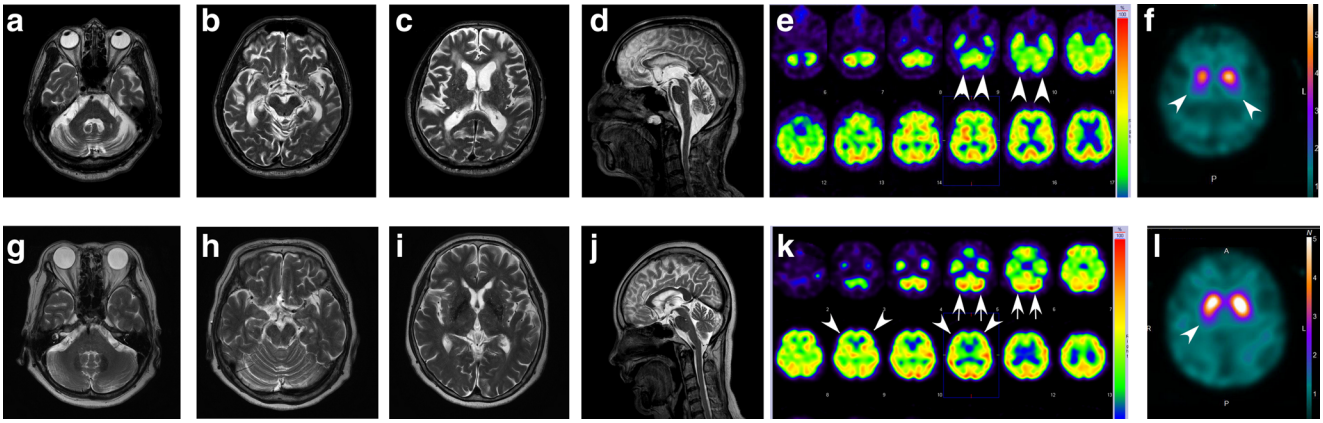

Fig. 4 The result of imaging in a patient who had PSP with 131 combined repeats (**a**–**f**) and a patient who had ataxia with intermediate (74) repeats (**g**–**l**). **a**–**d** Brain MRI T2-weighted imaging (GE, SIGNA 3T, TE/TR = 80/4000 [a sagittal image] and TE/TR = 100/5661.07 [axial images]) showed modest cerebellar atrophy and mild atrophy of the midbrain and the frontal lobes with multiple ischemic changes in the white matter, the basal ganglia, and the brainstem. **e** IMP-SPECT revealed decreased perfusion in the cerebellum (arrowhead). **f** Dopamine transporter imaging using ^{123}I-ioflupane revealed severely reduced tracer uptake in bilateral putamen (arrowhead). **g**–**j** Brain MRI T2-weighted imaging (GE, SIGNA 3T, TE/TR = 80/4000 [a sagittal image] and TE/TR = 100/5661.07 [axial images]) showed very mild cerebellar atrophy without apparent abnormality of the frontal lobes, the basal ganglia, or the brainstem. **k** IMP-SPECT revealed decreased perfusion in the bilateral frontal lobes (arrowhead), with preserved perfusion in the cerebellum (arrow). **l** Dopamine transporter imaging using ^{123}I-ioflupane revealed mildly reduced tracer uptake in bilateral putamen with a right-predominant reduction (arrowhead)

showed slight cerebellar atrophy (Fig. 5). His symptoms gradually progressed; he visited our hospital at the age of 77 years. Neurologic examinations revealed mild dementia, vertical gaze paresis, dysphagia, dysarthria, neck dystonia, right-hand tremor at rest, mild-to-moderate rigidity in all limbs, severe postural instability, and inability to walk. His deep tendon reflex remained unaltered with bilateral extensor plantar responses. Levodopa responsiveness was not apparent at that time. His MRI image showed modest atrophy in the midbrain and mild atrophy in the cerebellum (a series of brain MRIs is shown in Fig. 5). Unfortunately, IMP-SPECT was declined. Dopamine transporter imaging or MIBG scintigraphy was unavailable in Japan during his examination. He had an ileus of unknown cause and underwent surgery. He was then lost to follow-up. Based on the clinical diagnostic criteria, his condition was diagnosed as probable PSP–Richardson syndrome (RS) [26].

A Patient with an Intermediate Repeat

Detailed information of a 76-year-old woman with an intermediate repeat was described in the supplemental document.

Functional Imaging Results

The results of functional imaging, such as cerebral perfusion SPECT, MIBG scintigraphy, and dopamine transporter imaging, were available for five patients, including the present and reported patients (Table 1). Representative findings are shown in Fig. 4. Cerebral perfusion SPECT result showed two patients with decreased cerebral perfusion in the cerebellum and two with decreased cerebral perfusion in the frontal lobes. Dopamine transporter imaging showed that all three patients examined had decreased putaminal uptake, irrespective of their phenotypes. Three of the patients had a normal MIBG scintigraphy result.

Results of Analyses in Unaffected Individuals

The supplemental Fig. 1 shows that only approximately 20% of unaffected individuals were older than the oldest age of patients with similar repeat sizes.

Discussion

This study clarified that two patients with PSP had mutations in the *ATXN8OS* gene. In addition, parkinsonism, as found in patients with Parkinson's disease or related disorders, including PSP, was associated with shorter repeat expansions in the *ATXN8OS* gene than predominant ataxia. Such repeat-related phenotypic differences have been reported in several other triplet repeat diseases. In DRPLA, shorter repeat sizes are associated with ataxia with choreic movements, while longer repeat sizes are associated with myoclonus epilepsy [27]. In SCA2, shorter or intermediate repeat sizes are associated with amyotrophic lateral sclerosis [28], while longer repeat sizes are associated with ataxia. Predominant ataxia without parkinsonism in one of our patients with an intermediate repeat size and overlapped repeat sizes seen in both phenotypes suggest that repeat sizes do not completely determine phenotype. Nonetheless, the dopamine transporter image revealed that the patient had reduced tracer uptake in bilateral putamen, a similar finding of our patient with parkinsonism, indicating

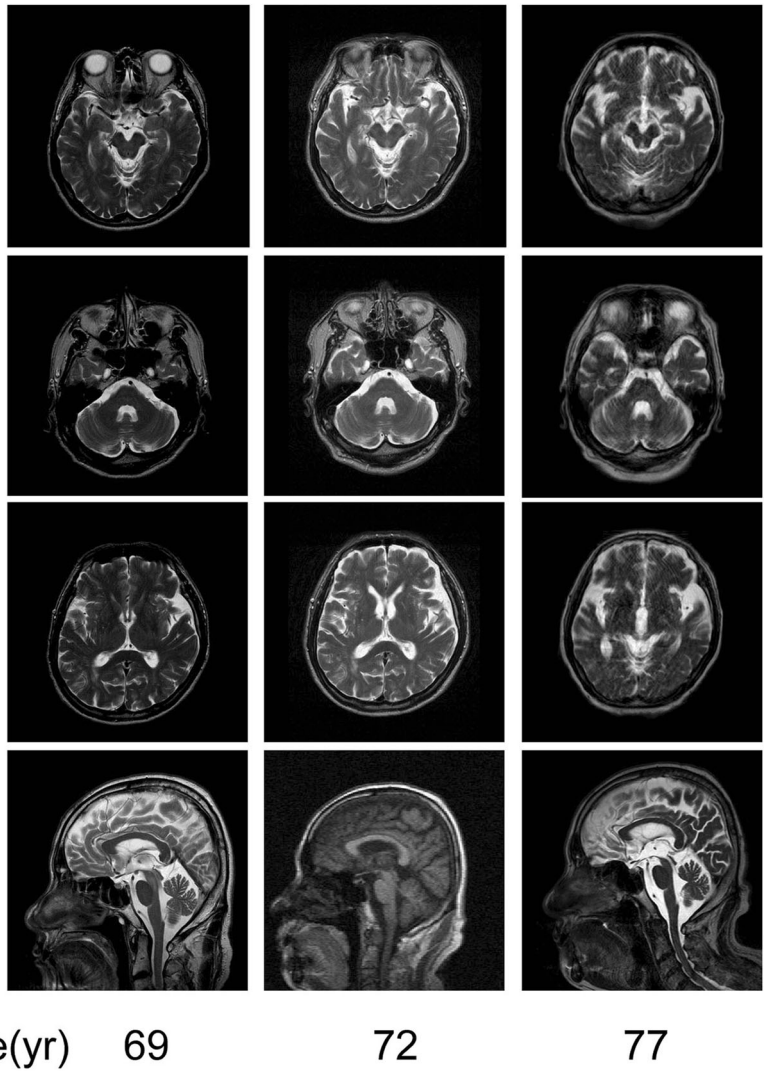

Fig. 5 A series of MRI for a patient who had PSP with 93 combined repeats. MRI at the age of 69 years (2 years after onset) showed slight atrophy of the cerebellum. MRI at the age of 72 years showed mild atrophy of the cerebellum and frontal lobes. MRI at the age of 77 years showed modest atrophy of the midbrain and frontal lobes and mild atrophy of the cerebellum. Only a scout view was available as sagittal MRI at the age of 72 years

that a presymptomatic abnormality in the dopamine metabolism had started. Other factors should be associated with manifestation of symptoms. The closer relationship between older age at onset and a parkinsonism phenotype than that between shorter repeat sizes and the phenotype suggested that aging is associated with parkinsonism.

The imaging result of SCA8 has rarely been reported, whereas that of PSP has been mostly established. Our preliminary result of imaging analyses in our patients and those reported in patients with SCA8 revealed decreased cerebral perfusion on SPECT in the frontal lobes in two patients with SCA8, which is a finding that is commonly observed in PSP [29]. Other patients had decreased cerebral perfusion in the cerebellum, which is a finding that is commonly observed in several types of ataxia. Notably, such locations did not match the phenotypes. In contrast, dopamine transporter imaging showed decreased tracer uptakes in all three patients examined, with more prominent decreases in the parkinsonism phenotype. In our results, no patients had decreased MIBG uptake. The positive result of dopamine transporter imaging together with the negative result of MIBG scintigraphy was consistent with the imaging features of PSP or atypical parkinsonian disorders.

We have found for the first time a significant inverse correlation between age at onset and CTA/CTG repeat sizes in all patients with SCA8, despite such a correlation being unestablished in previous reports [8, 9]. Thus, previous cohorts may not have been large enough to detect statistical significance. Our result suggests that larger repeat sizes may be associated with more toxic effects, as proposed for other triplet repeat diseases, such as SCA1, SCA2, SCA3, SCA6, SCA17, and DRPLA. The pathomechanism of SCA8 may therefore share at least partly that in other triplet repeat diseases. Notably, the age at onset of patients with ≥ 200 repeats tended to be more than expected; therefore, limiting the repeat size to < 200 provided a closer relationship. This finding suggests that ≥ 200 repeats is likely to be associated with a different pathomechanism that involves lower toxicity. In another

Table 1 Functional imaging results of patients with SCA8

References	Present report	Present report	Present report	Rinsho Shinkeigaku 2013; 53:278	J Clin Neurol 2013; 9:274–279
Case no.	1	2	2		
Sex	M	M	F	M	M
Age at onset	76	74	30	29	43
Age at examination	77	76	32	31	51
Symptoms	B, R, P	AT	AT	RT, R, B, P, LR	B, R, P, LR, AT
Repeat configuration	(CTA)10 (CTG)121	(CTA)16(CTG)6 CTA(CTG)51	(CTA)7 (CTG)158	nd	nd
CTA/CTG number	131	74	165	141	103
Site of decreased blood flow on SPECT	Bil. Cerebellum	Frontal lobes	Bil. Cerebellum	Frontal lobes	nd
MIBG uptake	Normal	Normal	nd	Normal	nd
DaT imaging	Decreased	Mildly decreased	nd	nd	Decreased

RT resting tremor, *B* bradykinesia, *R* rigidity, *P* postural instability, *LR* levodopa responsiveness, *AT* ataxia, *ANS* apparent autonomic dysfunction, *nd* not described, *SPECT* single photon emission computed tomography, *MIBG* 3(meta)-iodobenzylguanidine scan, *DaT* dopamine transporter

non-coding triplet repeat disease, fragile X syndrome, alleles with > 200 repeats are transcriptionally silent [16, 30]. We speculate that *ATXN8OS* transcription with ≥ 200 repeats may be at least partly suppressed, thereby reducing the toxicity, although it may still retain pathogenicity. Our findings suggest that larger repeat sizes up to 200 repeats may be associated with more toxic effects; however, further studies are required to draw definitive conclusions.

We also analyzed unaffected individuals with repeat expansions in the *ATXN8OS* gene. Available data suggests that only approximately 20% of those individuals surpassed the oldest ages for patients with equivalent repeat sizes, suggesting that several unaffected individuals might come to be affected later in life. Such a repeat-related age at onset might account for the reported finding that shorter repeats are associated with low penetrance because when a given age is set for the inclusion criteria in a cohort, for example 50 years, more individuals with shorter repeats may not reach the onset ages. It could be asserted that extremely long repeats, such as > 1000, were more commonly found in unaffected individuals. However, the present review indicates that such long repeats were found in both affected and unaffected individuals; however, there is no detailed clinical information [9].

In patients with SCA8, the rate of homozygotes or compound heterozygotes is thought to be higher than in other triplet repeat diseases, including spinocerebellar ataxia type 6, where dosage effects of expanded repeats have been at least partly suggested [31]. Interestingly, when we replaced larger repeat sizes with mean repeat sizes of longer and shorter expansions, a closer relationship was observed between age at onset and repeat size. In contrast, sum of expanded allele sizes did not improve the correlation. In addition, our result revealed that homozygotes or compound heterozygotes had no significantly earlier age at onset than heterozygotes with equivalent repeat sizes. These data may suggest that dosage effects of the expanded repeat are not apparent in patient with SCA8. Some undetermined genetic or environmental factors may be implicated in the onset of this disease.

Many pathomechanisms of SCA8 have been proposed, and some of them have been associated with repeat sizes. Typically, polyglutamine toxicity has been significantly correlated with repeat sizes. In RNA toxicity, CTG (CUG) repeat size is also a factor in determining the number of foci per nucleus and fraction of foci-positive nuclei in muscles of patients with myotonic dystrophy type 1 (MD1), another disease associated with CTG repeat [32]. However, such an increase was observed in muscles with a repeat size of 1250–1900 in patients with MD1, a finding with a large difference in repeat sizes as compared to SCA8. In contrast, the toxicity of repeat-associated non-ATG translation was not clearly associated with repeat sizes [33]. Our present data also suggest that various pathomechanisms are involved, which may be partly dependent on repeat sizes.

In summary, we found a significant relationship of CTA/CTG repeat sizes in patients with SCA8 with phenotype and age at onset. A limitation of this study is that we analyzed patients from different countries and races together, which could have introduced an element of bias. To avoid such a bias, we also analyzed only Japanese patients, and in doing so, we similarly found a significant relationship. Another limitation is that we did not analyze the genes that are causative for rare types of autosomal dominant or recessive ataxia to exclude additional mutations. However, most of the major genes associated with ataxia and PSP and some of the rare genes were analyzed in this study, and no mutation was detected in these genes. Future studies including whole genome sequencing may detect novel or known variations to develop or modify the phenotype for SCA8. The coincidental

occurrence of SCA8 and PSP is possible, but their relatively low prevalence in Japan (0.7/100,000 and 6/100,000) suggests that this condition is unlikely to happen in two unrelated patients. Our data showed that age at onset in similar repeat sizes varies considerably, which suggests that careful application is needed in genetic counseling. However, our results may be still useful to genetic counseling, improve understanding the pathomechanism, and extend clinical phenotype of SCA8.

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Author Contributions 1. Design or conceptualization of the study.
2. Acquisition of the data.
3. Analysis or interpretation of the data.
4. Drafting or revising the manuscript for intellectual content.
Dr. Makoto Samukawa has contributed to 1, 2, and 3.
Dr. Makito Hirano has contributed to 2, 3, and 4.
Dr. Kazumasa Saigoh has contributed to 2, 3, and 4.
Dr. Shigeru Kawai has contributed to 2, 3, and 4.
Dr. Yukihiro Hamada has contributed to 2, 3, and 4.
Dr. Daisuke Takahashi has contributed to 2, 3, and 4.
Dr. Yusaku Nakamura has contributed to 1, 3, and 4.
Dr. Susumu Kusunoki has contributed to 1, 3, and 4.

Compliance with Ethical Standards

Conflict of Interest None of the authors have any conflict of interest, including financial, personal, or other relationships with other people or organizations within 3 years since beginning the work submitted that could inappropriately influence (bias) our work.

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