Letters to the editors

Title: *FGF14* GAA repeat expansion and *ZFHX3* GGC repeat expansion in clinically diagnosed multiple system atrophy patients

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Statement and Declarations

Competing Interest

There were no financial, consulting, or personal relationships with other people or organizations that could influence our study.

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Ethic Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. informed consent was obtained for all participants and the study was approved by the institutional review boards of Hokkaido University Hospital and Yokohama City University Faculty of Medicine.

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53 *Fibroblast Growth Factor 14 (FGF14)* is the causative gene of spinocerebellar
54 ataxia 27B (SCA27B), in which abnormal GAA repeat expansion has been observed [1].
55 SCA27B is a late-onset autosomal dominant spinocerebellar degeneration characterized
56 by episodic ataxia and nystagmus [2]. More than 250 GAA repeat expansions have been
57 reported in *FGF14* [1]. GAA repeat expansion in *FGF14* is noteworthy, as it may be
58 buried in sporadic ataxia. Contrastingly, multiple system atrophy (MSA) is a rapidly
59 progressive intractable neurodegenerative disease characterized by autonomic
60 dysfunction, levodopa-unresponsive parkinsonism, and cerebellar ataxia [3]. Recently,
61 several studies have been conducted to establish disease-modifying therapies for MSA
62 [4]. To date, we have treated several patients with MSA, investigated the number of
63 *FGF14* repeat expansions, and analyzed their clinical trends. We believe that
64 investigating *FGF14* abnormalities in MSA will further help develop disease-modifying
65 therapies for MSA in the future. In addition, SCA4 is a rare autosomal dominant
66 spinocerebellar degeneration, and a GGC repeat expansion in the *Zinc Finger Homeobox*
67 *3 (ZFHX3)* was recently identified as the causative gene [5]. In SCA4, it was mentioned
68 that cerebellar ataxia was accompanied by sensory neuropathy, but the paper also reported
69 that the patients also presented with autonomic dysfunction, motor neuropathy, slow

ocular saccades, dysphagia, and dystonia. From the perspective of cerebellar ataxia accompanied by autonomic dysfunction, it is necessary to consider SCA4 as a differential disease for MSA, and we also investigated the presence or absence of abnormalities in the *ZFHX3* gene.

Patients with MSA who underwent DNA analysis from 1993 to 2022 at our department were included. MSA diagnosis was assessed according to the second consensus statement (2008) [6]. The presence or absence of abnormal GAA repeat expansion in *FGF14* and GGC repeat expansion in *ZFHX3* was examined in the conserved DNA. First, the GAA repeat expansion in *FGF14* was confirmed via amplification using repeat-primed PCR and agarose gel electrophoresis. Moreover, in cases where abnormal expansions were suspected, detailed repeat analysis was performed for PCR-positive cases using a nanopore GridION sequencer (Oxford Nanopore Technologies, Oxford, UK) with an adaptive sampling option as previously described [7]. The GAA repeat unit count was determined using RepeatAnalysisTools (https://github.com/PacificBiosciences/apps-scripts/tree/master/RepeatAnalysisTools) after generating the consensus sequence of the expanded repeat using lamassemble (https://gitlab.com/mcfrith/lamassemble). In SCA27B, while GAA repeat lengths of ≥ 250 are usually considered positive [1, 2], cases of onset have been reported even with repeats < 250 [8, 9]. Herein, ≥ 200 GAA repeats

were considered pathological. The same study was conducted in a healthy control group at our department. A similar method was used for SCA4 to confirm the presence or absence of GGC repeat expansion in *ZFHX3*. In accordance with previous reports [5], a *ZFHX3* GGC repeat count of 42 or more was considered pathological. We used information from the medical records of our department and the Hokkaido Rare-Disease Consortium for Multiple System Atrophy (HoRC-MSA) [10], and retrospectively analyzed the data. Additionally, clinical information (sex, age at onset, initial symptoms, disease duration, symptoms on blood sampling, MRI findings, and diagnostic criteria) was confirmed for cases in which *FGF14* abnormalities were detected. We compared data from the HoRC-MSA and our department as disease controls. The conventional second consensus statement [6] was used as the diagnostic criterion since this study included a retrospective analysis and the clinical information required for the new diagnostic criteria [11] was insufficient. This study was approved by the Institutional Review Boards of our hospital (14-004) and Yokohama City University Faculty of Medicine. Written opt-out consent was obtained from all patients and their families.

We enrolled 411 patients with MSA (average age ± standard deviation at the time of blood collection: 64.9 ± 9.2 years), and GAA repeat expansion in *FGF14* was detected in two of them. GAA repeat expansion of *FGF14* was not observed in healthy controls (n =

200). Table 1 summarizes the clinical characteristics of the GAA repeat cases with *FGF14* abnormalities and disease controls. Of the 411 patients, 184 for whom clinical information could be clearly collected and who had already been reported in HoRC-MSA were treated as the disease controls. The *FGF14* abnormalities occurred in men in their 50s with possible MSA-C (cerebellar ataxia dominant) and women in their 70s with probable MSA-P (parkinsonism dominant) based on the second consensus statement criteria. In the MSA-C patient, ataxia was predominant, and slight parkinsonism was observed one year from onset; however, in addition to mild dysuria, orthostatic hypotension was not confirmed. Brain MRI at the first visit showed slight brainstem and cerebellar atrophy; however, the hot cross bun sign or putaminal abnormality was unclear (Figures 1A, 1B, 1C, and 1D). The patient was diagnosed with MSA-C. The GAA repeat unit count of the *FGF14* gene was 210. Subsequently, the symptoms progressed, and 5 years after the onset, the patient was bedridden and required urinary catheterization. The patient had orthostatic hypotension, stridor suggestive of vocal cord dysfunction, and sleep apnea. Lead-pipe rigidity was also observed; however, levodopa was not administered. Brain MRI showed that the brainstem and cerebellar atrophy had progressed, and the hot cross-bun sign became clear (Figures 1E, 1F, 1G, and 1H). The patient suddenly died and an autopsy was not available. The patient had no sputum retention and was normally asleep the night

124 before death. The patient was found the next morning without vital signs and was
125 confirmed dead. In the MSA-P patient, parkinsonism accompanied by autonomic
126 dysfunction was observed at the first visit, 3 years after onset. MRI showed unclear
127 putaminal abnormality; however, atrophy of the cerebellum and pons and a less clear hot-
128 cross bun sign were confirmed. (Figures 1I, 1J, 1K, and 1L) and was diagnosed with
129 probable MSA. The GAA repeat unit count *FGF14* was 238. The patient's symptoms
130 continued to progress, and activities of daily living decreased. Several months later, the
131 patient was transferred to a long-term care hospital and the subsequent course was
132 unknown. On the other hand, the GGC repeat expansion in *ZFHX3* was 29 or less in all
133 of our cases, and the presence of SCA4 in clinically diagnosed MSA was ruled out within
134 the scope of our investigation.

135 This study had two important findings. First, *FGF14* may be a disease-associated gene
136 in MSA. Second, *FGF14* repeat expansion may be mixed in MSA. In SCA27B, the GAA
137 repeat expansion of *FGF14* was observed, and several cases of SCA27B are estimated to
138 be present in Japan [9, 12]. Typically, the nystagmus frequency is high in SCA27B [2,
139 12]. However, in our study, nystagmus was not evident in the *FGF14* repeat expansion
140 patients. The subsequent course of the *FGF14* repeat expansion cases was consistent with
141 MSA and different from that of SCA27B. The difference in nystagmus frequency was

142 considered the differentiation point between MSA and SCA27B. Recently, cerebellar
143 ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) has attracted
144 attention as a differential diagnosis of MSA [13]. CANVAS is an autosomal recessive
145 disease associated with the pentanucleotide expansion such as AAGGG in the *RFC1* gene.
146 CANVAS can present with clinical symptoms and imaging findings similar to those of
147 MSA; therefore, suspecting CANVAS and SCA27B when diagnosing MSA is necessary.
148 From the MSA perspective, patients with *FGF14* repeat expansion exhibited unclear hot-
149 cross bun signs or putaminal abnormalities, which differed from the symptoms of typical
150 MSA. This atypical trend may delay MSA diagnosis but also suggest MSA is a
151 heterogeneous disease. From another perspective, *FGF14* repeat expansion may affect
152 MSA pathogenesis. Although MSA is a sporadic disease, several disease-associated genes,
153 including *COQ2* and *GBA* have been reported [14, 15]. FGF14 is a member of the FGF
154 family and is involved in various biological processes such as embryogenesis, cell
155 proliferation, tissue repair, and tumor growth. If *FGF14* can be considered an MSA
156 disease modifier, it could be used to develop new treatments; therefore, further analysis
157 is required. However, the number of repeat expansions in the two patients in the present
158 study was slightly < 250. Since some previous reports suggest that SCA27B may develop
159 even with < 250 GAA repeats [8, 9], *FGF14* pathogenicity needs to be investigated in a

larger number of patients. This study has some limitations. First, this study was retrospective. There were cases where clinical information about MSA was insufficiently collected. Since this study used the second consensus statement, we could not deny the possibility that so-called MSA-like disease was included. It is desirable the new diagnostic criteria (the Movement Disorder Society criteria) should be used. Second, although MSA could be clinically recognized, an autopsy was not performed; therefore, MSA could not be pathologically confirmed. Third, determining whether the presence of *FGF14* repeat expansion in MSA is unique may be challenging because the frequency of *FGF14* repeat expansion in a large cohort of healthy controls and patients with other diseases is unknown. The two cases presented in this article differ from the classical SCA27B, and the GAA repeat expansion in *FGF14* may be a casual finding. To examine *FGF14* pathogenicity, confirming the frequency of *FGF14* repeat expansion in healthy control groups and in other diseases in the future is necessary. The influence of the 200-250 repeats of GAA in *FGF14* requires further accumulation of cases and some kind of functional analysis. For example, investigation using induced pluripotent stem cells derived from cases with GAA 200-250 repeats is considered one of the methods. Furthermore, although SCA4 needs to be considered as a differential diagnosis for MSA, it is still infrequent, and it is assumed that there are more cases in which it is not detected

178 even when searching for *ZFHX3*. However, *ZFHX3* may also be worth searching for if
179 there is a family history or if it is atypical MSA.

180 In conclusion, the GAA repeat expansion of *FGF14* was detected in patients clinically
181 diagnosed with MSA. In order to elucidate its pathological significance, it is necessary to
182 accumulate more cases and examine the interpretation of the influence of 200-250 GAA
183 repeats. It is also desirable to continue searching for the presence or absence of *ZFHX3*
184 abnormalities in MSA.

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225 **Figure legends**

226 Figure 1. Brain MRI of the pure GAA repeat expansion patient.

227 A, B, C, and D: At the first visit of the MSA-C patient E, F, G, and H: Five years after

228 MSA-C onset. I, J, K, and L: At the first visit for the MSA-P patient. A, E, and I.: sagittal

229 T1 weighted images. B, C, D, F, G, H, J, K, and L: axial T2 weighted images.

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232 Table 1. Characteristics of the *FGF14* repeat expansion patients and the control group

233 UMSARS, unified multiple system atrophy rating scale; ND, not described.

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236 **Author contribution**

237 Drafting of the manuscript: M-M

238 Patient medical treatment: M-M, A-K, T-M, S-U, A-K, and T-F

239 Genetic analyses: E-K and S-M

240 Data analysis: M-M and A-K

241 Manuscript review for intellectual content: H-Y, E-K, T-M, S-U, S-M, N-M, and I-Y.

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Data availability statement

Data will be available on request from the authors.

Table 1. Characteristics of the *FGF14* repeat expansion cases and the control group

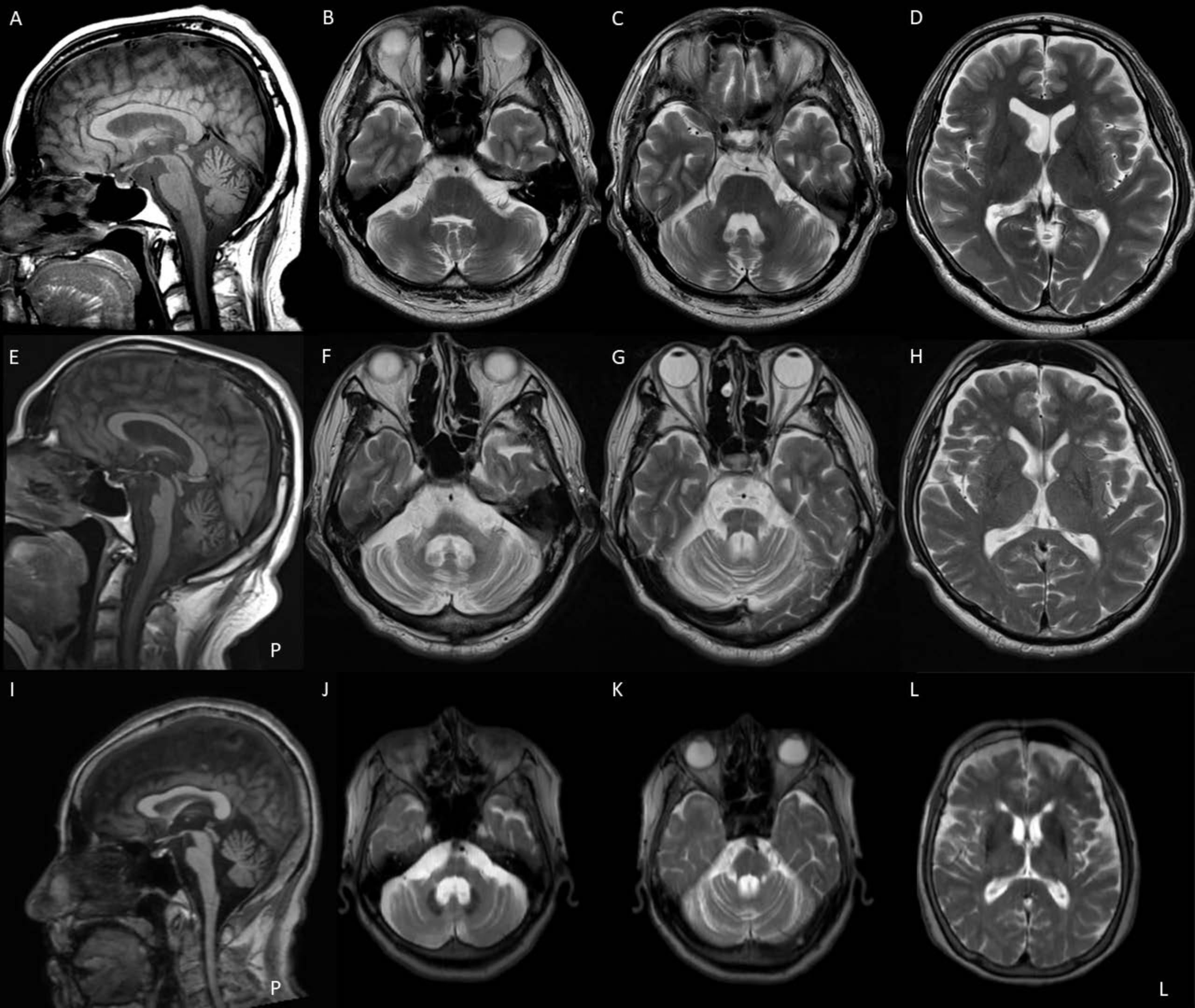
	FGF14 repeat expansion cases#		Control group (n = 184)*
Sex	male	female	53% females
Onset age	52	70	66.4 ± 10.9
Dominant symptom at onset	cerebellar ataxia	parkinsonism	58% cerebellar ataxia
Diagnostic criteria	possible	probable	76% probable
UMSARS part 4	2	4	3.4 ± 1.2
Symptoms at blood sampling			
Urinary dysfunction	+	+	**42%
Constipation	-	+	**16%
Orthostatic hypotension	-	+	**37%
Vocal cord dysfunction	-	-	**31%
Sleep apnea	-	-	ND
Parkinsonism	+	+	**76%
Cerebellar ataxia	+	+	**90%
Nystagmus	-	-	**35%
Cognitive impairment	-	+	ND
Levodopa unresponsiveness	Non-used	-	ND
MRI findings			
Cerebellar atrophy	+	+	81%
Brain stem atrophy	+	+	78%
Hot cross bus sign	±	±	69%
Putaminal abnormal signal	-	-	31%
GAA repeat length in *FGF14*	210	238	-

UMSARS: Unified Multiple System Atrophy Rating Scale; ND: not described

#: The findings were from the initial examination.

*: Data from Reference 9 and our department

**: symptoms at first evaluation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