Background: Multiple system atrophy (MSA) has been considered a sporadic disease, without patterns of inheritance.

Objective: To describe the clinical features of 4 multiplex families with MSA, including clinical genetic aspects.

Design: Clinical and genetic study.

Setting: Four departments of neurology in Japan.

Patients: Eight patients in 4 families with parkinsonism, cerebellar ataxia, and autonomic failure with age at onset ranging from 58 to 72 years. Two siblings in each family were affected with these conditions.

Main Outcome Measures: Clinical evaluation was performed according to criteria by Gilman et al. Trinucleotide repeat expansion in the responsible genes for the spinocerebellar ataxia (SCA) series and for dentatorubral-pallidoluysian atrophy (DRPLA) was evaluated by polymerase chain reaction. Direct sequence analysis of coding regions in the α-synuclein gene was performed.

Results: Consanguineous marriage was observed in 1 of 4 families. Among 8 patients, 1 had definite MSA, 5 had probable MSA, and 2 had possible MSA. The most frequent phenotype was MSA with predominant parkinsonism, observed in 5 patients. Six patients showed pontine atrophy with cross sign or slitlike signal change at the posterolateral putaminal margin or both on brain magnetic resonance imaging. Possibilities of hereditary ataxias, including SCA1 (ataxin 1, ATXN1), SCA2 (ATXN2), Machado-Joseph disease/SCA3 (ATXN1), SCA6 (ATXN1), SCA7 (ATXN7), SCA12 (protein phosphatase 2, regulatory subunit B, β isomorph; PP2R2B), SCA17 (TATA box binding protein, TBP) and DRPLA (atrophin 1; ATN1), were excluded, and no mutations in the α-synuclein gene were found.

Conclusions: Findings in these multiplex families suggest the presence of familial MSA with autosomal recessive inheritance and a genetic predisposition to MSA. Molecular genetic approaches focusing on familial MSA are expected to provide clues to the pathogenesis of MSA.

Arch Neurol. 2007;64:545-551

Multiple System Atrophy (MSA) is a sporadic neurodegenerative disorder characterized by various combinations of parkinsonism, cerebellar ataxia, and autonomic failure. The discovery of glial cytoplasmic inclusions (GCIs) established MSA as a distinct neurodegenerative disorder, and α-synuclein was found to be a major component of GCIs. Furthermore, α-synuclein in GCIs has been found to be abnormally phosphorylated at Ser129, and phosphorylated α-synuclein has been demonstrated to promote fibril formation in vitro. However, the etiology of MSA remains to be elucidated. Recently, familial cases of MSA with probable autosomal dominant inheritance were reported in Germany and Japan. Herein, we report findings in 4 multiplex families with MSA that suggest an autosomal recessive model of inheritance.

Methods

Clinical and Pathological Analysis

We identified 4 Japanese families, families A through D, in which multiple siblings were affected with MSA (Figure 1). The diagnosis of MSA was made on the basis of the consensus criteria for MSA by Gilman et al. Brain magnetic resonance (MR) imaging or computed tomography was performed in 7 patients; in the eighth patient (II-4 in family A), an autopsy was performed. Selected histopathological sections of 4-μm thickness were stained with hematoxylin-eosin, Klüver-Barrera Luxol fast blue, or Gallyas-Braak silver. Immunohistochemical analysis was conducted using an anti-α-synuclein–precursor of the non-Aβ compo-
nent of Alzheimer disease amyloid (NACP) antibody as previously described.8

**MOLECULAR GENETIC ANALYSIS**

Genomic DNA was extracted from peripheral blood leukocytes after obtaining informed consent. Expansions of CAG repeats of the genes for dominant spinocerebellar ataxias (SCAs), including SCA1 (ataxin 1; \textit{ATXN1} [for gene nomenclature see http://www.gene.ucl.ac.uk/nomenclature9]), SCA2 (ATXN2),10 Machado-Joseph disease/SCA3 (ATXN1),14 SCA6 (ATXN1),15 SCA7 (ATXN7),16 SCA12 (protein phosphatase 2, regulatory subunit B, β isofrm, PP2R2B),17 SCA17 (TATA box binding protein, \textit{TBP}),18,19 and dentatorubral-pallidoluysian atrophy (DRPLA [atrophin 1, \textit{ATN1}]),20,21 were analyzed using an automated DNA sequencer (ABI 377; Applied Biosystems, Foster City, Calif) in the affected patients. Direct sequence analysis of 6 coding exons of the α-synuclein (\textit{SNCA}) gene was performed using the ABI 3100 DNA sequencer in all patients.

**RESULTS**

Two siblings were affected in each family. The parents of siblings in each family had no clinical signs of extrapyramidal or cerebellar disorders. Consanguineous marriage was present in family A, in which the parents were first-degree cousins. Among 8 patients, 1 (II-4 in family A) had definite MSA, 5 (II-8 in family A, II-2 and II-9 in family B, II-5 in family C, and II-7 in family D) had probable MSA, and 2 (II-4 in family C and II-3 in family D) had possible MSA. The most frequent phenotype was MSA with predominant parkinsonism (MSA-P), observed in 5 patients. One patient showed an MSA of the cerebellar type (MSA-C) phenotype, and 2 patients showed an MSA-P + C phenotype. The clinical phenotypes were concordant between the affected siblings in the 3 families (families A, B, and C). The mean age at onset was 65.9 years (age range, 58-72 years). Six patients showed pontine atrophy with cross sign or slitlike signal change at the posterolateral putaminal margin or both on brain MR imaging. Clinical features of 4 multiplex families with MSA are summarized in the Table.

Testing for the trinucleotide repeat expansions in the responsible genes for SCA1 (ATXN1), SCA2 (ATXN2), Machado-Joseph disease/SCA3 (ATXN1), SCA6 (ATXN1), SCA7 (ATXN7), SCA12 (PP2R2B), SCA17 (TBP), and DRPLA (ATN1) gave normal results. No mutations in the \textit{SNCA} gene were found in the family members.

**REPORT OF CASES**

**FAMILY A**

Patient II-4 was diagnosed as having retinitis pigmentosa at age 33 years by an ophthalmologist. She noticed resting tremor in her left hand at age 68 years, followed by the development of bradykinesia and gait disturbance at age 69 years. Neurological examination at age 71 years revealed bradykinesia, a masklike face, urinary frequency, severe rigidity in the 4 limbs, and limb ataxia predominant on the left side. Parkinsonism was not improved by levodopa treatment. She did not show dementia, muscular atrophy, sensory disturbance, or limitation of ocular movement. Brain computed tomography showed distinct cerebellar atrophy. Brain MR imaging was not performed. She had recurrent pneumonia and died at age 73 years. On postmortem examination, the brain weighed 1030 g before fixation. On gross examination, the cerebellum and pontine base were moderately atrophic. The inferior olivary nuclei were slightly atrophic. Moderate depigmentation in the substantia nigra and locus coeruleus was observed. Microscopically, moderate to severe loss of neurons with gliosis was observed in the pontine nuclei. The transverse bundles in the pontine base
showed severe loss of myelinated nerve fibers. The loss of Purkinje cells was mild to moderate, and the cerebellar white matter was severely degenerated. In the cerebellar dentate nucleus, neuronal shrinkage associated with gliosis was observed. Moderate neuronal loss associated with gliosis was observed in the substantia nigra and locus coeruleus. In the putamen, a small amount of GCIs was observed, but no obvious neuronal loss was observed. Mild neuronal loss was also detectable in the red nucleus and dorsal vagal and vestibular nuclei. In the thalamic medial nuclei, gliosis without distinct neuronal loss was observed. Numerous argyrophilic GCIs and an abnormal accumulation of $\alpha$-synuclein–precursor of the non-$\alpha$ component of Alzheimer disease amyloid (NACP) in glial cells were observed in the substantia nigra, pontine base, inferior olive, and cerebellar white matter (Figure 2). In the retina, severe loss of rods and cones and patchy disappearance of pigmentary epithelial cells were observed.

**Patient II-8**

Patient II-8 developed night blindness at age 48 years and was diagnosed as having retinitis pigmentosa at age 51 years. He had mild resting tremor in both hands, bradykinesia, and rigidity at age 62 years, which were symmetrical and refractory to a combined therapy of 200 mg of levodopa–doparadecarboxylase inhibitor. He had difficulty in urination at age 63 years, orthostatic hypotension at age 64 years, and truncal ataxia at age 65 years. He became bedridden at age 66 years because of the progression of his symptoms. On laboratory investigation, his protein level, cell counts, glucose concentration, IgG index, and 5-hydroxyindoleacetic acid level in cerebrospinal fluid were within normal ranges. His homovanillic acid level was decreased to 19.9 ng/mL (reference range, 28-77 ng/mL). Thyroid hormone levels, serum and urinary copper levels, serum ceruloplasmin level, lysosomal enzyme activities in leukocytes, and vitamin B$_6$, B$_12$, and E levels were normal. Nerve conduction studies revealed no abnormalities. Brain MR imaging showed mild atrophy of the...
pons and cerebral cortex on T1-weighted imaging. Atrophy of the pontine base accompanied by cross sign on T2-weighted and proton density–weighted imaging was observed. Slitlike signal changes with hyperintensity on T1-weighted imaging and hypointensity on T2-weighted imaging were observed in the posterolateral putaminal margin (Figure 3A). Single-photon emission computed tomography images showed mild hypoperfusion in the pons, basal ganglia, and right frontal and left parietal lobes.

Both affected siblings in family A had retinitis pigmentosa. The other siblings were unaffected.

**FAMILY B**

**Patient II-2**

Patient II-2 began to have resting tremor in the right hand at age 72 years, followed by rigidity in the 4 extremities, parkinsonian gait, urinary incontinence, and orthostatic hypotension within a year. These symptoms responded poorly to a combined therapy of 900 mg of levodopa–dopadecarboxylase inhibitor. He required support in walking at age 76 years. Brain MR imaging revealed slitlike signal change at the posterolateral putaminal margin on T2-weighted imaging and pontine atrophy on T1-weighted imaging (Figure 3B).

**Patient II-9**

The brother of patient II-2, patient II-9, developed resting tremor in the right hand at age 63 years and in the right leg at age 65 years. Neurological examination at age 66 years revealed severe rigidity in the 4 extremities, bradykinesia, mild limb ataxia, urinary incontinence, and orthostatic hypotension. The symptoms responded poorly to a combined therapy of 900 mg of levodopa–dopadecarboxylase inhibitor. Brain MR imaging showed slitlike signal change at the posterolateral putaminal margin, atrophy of the cerebellar vermis, and cross sign in the pons on T2-weighted imaging (Figure 3C).

**FAMILY C**

**Patient II-4**

Patient II-4 had cerebellar ataxia at age 68 years, followed by the development of resting tremor predominantly on the left side, neck rigidity, parkinsonian gait, and urinary frequency. These symptoms were progres-
sive and responded poorly to a combined therapy of 300 mg of levodopa–dopacarboxylase inhibitor. She eventually required a wheelchair at age 73 years. Brain MR imaging performed at age 72 years showed no abnormalities.

Patient II-5

The brother of patient II-4, patient II-5, had resting tremor in the left hand, dysarthria, and urinary frequency at age 67 years. These symptoms progressed slowly. He further developed rigidity predominantly on the left side and truncal ataxia. He became wheelchair bound at age 70 years. Brain MR imaging showed severe cerebellar atrophy and pontine atrophy accompanied by cross sign (Figure 3D).

FAMILY D

Patient II-7

Patient II-7 had impotence and constipation at age 58 years and nocturnal urinary frequency at age 60 years. He had cerebellar ataxia at age 62 years and orthostatic hypotension at age 63 years. On physical examination at age 63 years, he showed prominent autonomic dysfunctions, including orthostatic hypotension, urinary frequency, constipation, and impotence. Moderate cerebellar ataxia, pyramidal signs such as Babinski sign with hyperreflexia, and extrapyramidal signs such as rigidity in the neck and upper limbs were also observed. Brain MR imaging showed severe atrophy of the cerebellum and pontine base accompanied by cross sign in the pons on proton density–weighted imaging (Figure 3E) and slitlike signal change at the posterolateral putaminal margin on T2-weighted imaging.

Patient II-3

The sister of patient II-7, patient II-3, had bradykinesia at age 69 years. Neurological examination at age 72 years revealed severe parkinsonism with poor response to levodopa therapy, hyperreflexia of the 4 limbs, and urinary incontinence.

Brain MR imaging showed severe atrophy of the cerebellum and the pons and slitlike signal change at the posterolateral putaminal margin on T2-weighted imaging. The clinical phenotypes were not concordant between the affected siblings in this family.

COMMENT

The 8 patients in 4 families were considered affected with MSA for the following reasons. First, all the affected members fulfilled the consensus criteria for the diagnosis of MSA according to Gilman et al except for family history of a similar disorder. Because it is important to differentiate MSA from progressive supranuclear palsy and from corticobasal degeneration, the consensus guide-

lines comprise exclusion criteria (including prominent slowing of vertical saccades, vertical supranuclear gaze palsy, and evidence of focal cortical dysfunction [such as aphasia, alien limb syndrome, and parietal dysfunction]) to increase the specificity of the diagnosis of MSA. None of the affected members fulfilled these exclusion criteria. Furthermore, none of the affected members fulfilled the clinical criteria for progressive supranuclear palsy or corticobasal degeneration. Second, 6 of 8 patients showed pontine atrophy with cross sign or slitlike signal change at the posterolateral putaminal margin or both on brain MR imaging. These findings have been established as radiological evidence for MSA, although such findings have been described infrequently in other diseases, including SCA2, Machado-Joseph disease, progressive supranuclear palsy, and corticobasal degeneration. In the families in the present study, SCA2 and Machado-Joseph disease were excluded by molecular diagnosis. Third, the possibilities of SCA12 and SCA17 mimicking parkinsonism and ataxia were excluded by molecular diagnosis as well.

Although MSA has been considered a sporadic neurodegenerative disease, a genetic predisposition to MSA was first described by Nee et al in 1991. They found higher frequencies of neurological diseases and autonomic symptoms among 148 first-degree relatives of 33 patients clinically diagnosed as having MSA compared with control subjects. Wenning et al reported a higher frequency of parkinsonism among first-degree or second-degree relatives of 38 autopsy-proven patients with MSA. Furthermore, a patient with pathologically proven MSA whose mother had autopsy-proven Parkinson disease was also described. Recently, 2 families with probable MSA in Germany and Japan have been described; these 2 families each have 2 affected individuals, suggesting autosomal dominant inheritance. In the families in the present study, consanguineous marriage was observed in family A, and no affected individuals were ascertained in successive generations, suggesting a hypothesis of autosomal recessive inheritance. The segregation ratio of 0.129 (95% confidence interval, 0.011-0.247) was estimated based on data from all 4 families. Although the ratio was slightly lower than 0.25, which is expected for an autosomal recessive inheritance model, the distribution of affected individuals in these families might be consistent with that expected for an autosomal recessive inheritance trait considering an ascertainment bias for multiplex families (χ² = 1.53, P = .22). We need additional multiplex MSA families to formulate any conclusive interpretation on mode of inheritance among our families. A second hypothesis on the genetic model for these families is that MSA susceptibility genes strongly contribute to the pathogenesis among these families. A third hypothesis is that 2 siblings in each family were affected by chance. Because the standardized prevalence rates of MSA are estimated to be 1.9 to 4.9 cases per 100,000 people, the possibility of finding 2 patients with MSA in a family of 10 or 12 members by chance is below 0.00006. Therefore, incidental occurrence of MSA in a single family is extremely rare.

We can identify some characteristic features of these families. First, most patients had the MSA-P phenotype.
This is inconsistent with the fact that the MSA-C phenotype is the most prevalent form of MSA in Japan. According to a recent nationwide survey in Japan, 82.5% of patients with MSA have the MSA-C phenotype, while 10.6% have the MSA-P phenotype. Second, the clinical phenotypes were concordant among the affected siblings in 3 of 4 families. Third, the mean age at onset (65.9 years) of these family members is later than that among patients with sporadic MSA (age range, 55.4-57.5 years). Fourth, pathological changes were prominent in the substantia nigra, while those in the putamen were very mild in patient II-4 in family A, despite the MSA-P phenotype. Because a high degree of neuronal loss in the putamen has been generally observed in subjects with the MSA-P phenotype, this observation is interesting. More pathologically proven cases of familial MSA are required to reveal the pathological features of familial MSA.

Because the SNCA gene is the major component of GCIs and of neuronal cytoplasmic inclusions in MSA, we considered this a candidate gene and conducted mutational analysis of the coding regions but failed to find any mutations in our patients. A previous study demonstrated no significant changes in the expression levels of the SNCA gene messenger RNA in the brains of patients with MSA compared with those of control subjects. Taken together, the SNCA gene is unlikely to be the causative gene for MSA. Other genetic or environmental factors associated with SNCA might be operable in the pathogenesis of MSA. To identify genetic factors related to MSA, investigations of additional multiplex families with MSA are necessary.

Accepted for Publication: October 27, 2006.

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Financial Disclosure: None reported.

Funding/Support: This study was supported by a Grant-in-Aid for Scientific Research on Priority Areas (C)–Advanced Brain Science Project and by a Grant-in-Aid for Scientific Research (A) from the Japan Ministry of Education, Culture, Sports, Science and Technology; by a Research for the Future Program grant from the Japan Society for the Promotion of Science; by a grant for Surveys and Research on Specific Diseases from the Japan Ministry of Health, Labor and Welfare; by a grant from the Virtual Research Institute of Aging from Nippon Boehringer Ingelheim; and by a grant from the Naito Foundation.

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