

ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Мултисистемна атрофия – церебеларен тип
Определение на заболяването
Мултисистемна атрофия церебеларен тип е форма на мултисистемната атрофия с преобладаващи церебеларни симптоми (динамична и локомоторна атаксия, окуломоторна дисфункция и дизартрия).
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G90.3
Код на заболяването по Orpha code
ORPHA227510
Епидемиологични данни за заболяването в Република България
Предполага се болестност около 1-9 / 100 000 за МСА общо, от които около 1/3 с МСА-церебеларен тип, подобна на тази в останалите страни в Европа.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. The Multiple-System Atrophy Research Collaboration. Mutations in COQ2 in familial and sporadic multiple-system atrophy. New Eng. J. Med. 369: 233-244, 2013. Note: Erratum: New Eng. J. Med. 371: 94 only, 2014. 2. Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., Vidailhet, M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670-676, 2008.
Епидемиологични данни за заболяването в Европейския съюз
1-9 / 100 000; В западното полукълбо около 1/3 общо от пациентите с МСА са с МСА-церебеларен тип. Разпределението е еднакво по пол.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. The Multiple-System Atrophy Research Collaboration. Mutations in COQ2 in familial and sporadic multiple-system atrophy. New Eng. J. Med. 369: 233-244, 2013. Note: Erratum: New Eng. J. Med. 371: 94 only, 2014. 2. Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., Vidailhet, M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670-676, 2008.
Оценка на съответствието на заболяването с дефиницията за рядко заболяване

съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето
Заболяването е с разпространение под 5 на 10 000 души от населението на Европейския съюз.
Критерии за диагностициране на заболяването
Диагностициране на заболяването (дефиниция на случай):
Признаците и симптомите на заболяването: МСА е с начало в зряла възраст обичайно между 55 до 60 години. Локомоторната атаксия е най-типичният начален симптом на МСА-церебеларен тип. Автономните нарушения (ранна уринарна инконтиненция, ортостатична хипотония, констипация, Raynaud синдром) се появяват рано и са задължителни за диагнозата МСА-церебеларен тип. Допълнителни черти за МСА-церебеларен тип са дисфония, дисфагия, други церебеларни черти (включва динамична атаксия) и окуломоторна дисфункция (sustained gaze-evoked nystagmus, positional down-beat nystagmus). Всички пациенти развиват в хода на заболяването поне някои паркинсонови черти (брадикинезия, ригидност, нерегулярен миоклоничен постурален тремор). Пирамидни белези (генерализирана хиперрефлексия и положителен Бабински) могат също да бъдат наблюдавани. Респираторни нарушения (сънна апнея, стридор и инспираторен и inspiratory sighs), нарушения в нощния сън (включващи REM-behavior disorder (RBD) и периодични движения на крайници по време на сън (PLMS) са често наблюдавани.
Етиологията и патогенезата: Точната етиологията на МСА е неизвестна, но наличието на цитоплазмени натрупвания на α -синуклеин, предимно в олигодендроглията, в комбинация с преобладаваща невродегенерация в оливопонтocereбралните структури са патологичен отличителен маркер на МСА-церебеларен тип. Мутации в COQ2 (4q21.23) (кодиращ ензима, участващ в биосинтеза на коензим Q10) са били установени в множество семейства с МСА, докато някои варианти се асоциират с повишен риск от спорадична МСА.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. Gilman, S., Low, P., Quinn, N., Albanese, A., Ben-Shlomo, Y., Fowler, C., Kaufmann, H., Klockgether, T., Lang, A., Lantos, P., Litvan, I., Mathias, C., Oliver, E., Robertson, D., Schatz, I., Wenning, G. Consensus statement on the diagnosis of multiple system atrophy. Clin. Auton. Res. 8: 359-362, 1998. 2. Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., Vidailhet, M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670-676, 2008. 3. Scholz, S. W., Houlden, H., Schulte, C., Sharma, M., Li, A., Berg, D., Melchers, A., Paudel, R., Gibbs, J. R., Simon-Sanchez, J., Paisan-Ruiz, C., Bras, J., and 26 others. SNCA variants are associated with increased risk for multiple system atrophy. Ann. Neurol. 65: 610-614, 2009. Note: Erratum: Ann. Neurol. 67: 277 only, 2010.
Алгоритми за диагностициране на заболяването
Алгоритми за диагностициране на заболяването: Диагнозата „вероятна“ МСА изисква наличието на церебеларни нарушения в съчетание с тежки автономни нарушения (необяснима уринарна инконтиненция или спадане на кръвното налягане при изправяне с поне 30 mm Hg систола или 15 mm Hg диастола). МРТ белези, включващи атрофия на средно малкомозъчно кряче. При [18F]-fluorodeoxyglucose PET се

установява хипометаболизъм на церебелум. „Дефинитивна” МСА изисква патоанатомично верифициране на α -синуклеин позитивни глиални цитоплазмени включвания с преобладаваща невродегенерация на оливоцеребеларните структури.

Анамнезата: МСА е с начало в зряла възраст обичайно между 55 до 60 години. Локомоторната атаксия е най-типичният начален симптом на МСА-церебеларен тип. Автономните нарушения (ранна уринарна инконтиненция, ортостатична хипотония, констипация, Raynaud синдром) се появяват рано и са задължителни за диагнозата МСА-церебеларен тип. Допълнителни черти за МСА-церебеларен тип са дисфония, дисфагия, други церебеларни черти (включва динамична атаксия) и окуломоторна дисфункция (sustained gaze-evoked nystagmus, positional down-beat nystagmus). Всички пациенти развиват в хода на заболяването поне някои паркинсонови черти (брадикинезия, ригидност, нерегулярен миоклоничен постурален тремор). Пирамидни белези (генерализирана хиперрефлексия и положителен Бабински) могат също да бъдат наблюдавани. Респираторни нарушения (сънна апнея, стридор и инспираторен и inspiratory sighs), нарушения в нощния сън (включващи REM-behavior disorder (RBD) и периодични движения на крайници по време на сън (PLMS) са често наблюдавани.

Диференциалната диагноза на заболяването: наследствени спиноцеребеларни атаксия (СЦА 1,2,3,6 и 7), чуплива-Х-хромозома асоцииран тремор/атаксия синдром (FXTAS) и митохондриопатии (POLG1 гена мутация).

Лабораторни, образни и хистологични изследвания: МСА церебеларен тип се характеризира патологично с преобладаваща дегенерация на оливопонтоцеребеларните структури и глиални цитоплазмени включвания, които се състоят от абнормно фосфорилиран α -синуклеин (SNCA) или тау (MAPT).

Генетични изследвания и медико-генетично консултиране: МСА-церебеларен тип се появява спорадично, но има описани някои фамилни случаи.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Gilman, S., Low, P., Quinn, N., Albanese, A., Ben-Shlomo, Y., Fowler, C., Kaufmann, H., Klockgether, T., Lang, A., Lantos, P., Litvan, I., Mathias, C., Oliver, E., Robertson, D., Schatz, I., Wenning, G. Consensus statement on the diagnosis of multiple system atrophy. Clin. Auton. Res. 8: 359-362, 1998.
2. Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., Vidailhet, M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670-676, 2008.
3. Scholz, S. W., Houlden, H., Schulte, C., Sharma, M., Li, A., Berg, D., Melchers, A., Paudel, R., Gibbs, J. R., Simon-Sanchez, J., Paisan-Ruiz, C., Bras, J., and 26 others. SNCA variants are associated with increased risk for multiple system atrophy. Ann. Neurol. 65: 610-614, 2009. Note: Erratum: Ann. Neurol. 67: 277 only, 2010.
4. Wullner, U., Abele, M., Schmitz-Heubsch, T., Wilhelm, K., Benecke, R., Deuschl, G., Klockgether, T. Probable multiple system atrophy in a German family. J. Neurol. Neurosurg. Psychiat. 75: 924-925, 2004.
5. Hara, K., Momose, Y., Tokiguchi, S., Shimohata, M., Terajima, K., Onodera, O., Kakita, A., Yamada, M., Takahashi, H., Hirasawa, M., Mizuno, Y., Ogata, K., Goto, J., Kanazawa, I., Nishizawa, M., Tsuji, S. Multiplex families with multiple system atrophy. Arch. Neurol. 64: 545-551, 2007.

6. The Multiple-System Atrophy Research Collaboration. Mutations in COQ2 in familial and sporadic multiple-system atrophy. <i>New Eng. J. Med.</i> 369: 233-244, 2013.
Алгоритми за лечение на заболяването
<u>Алгоритми за лечение на заболяването:</u> Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, а на когнитивните нарушения съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, както и съгласно международните критерии.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015. 3. Santiago Perez-Lloret, Olivier Flabeau, Pierre-Olivier Fernagut, Anne Pavy-Le Traon, María Verónica Rey, Alexandra Foubert-Samier, Francois Tison, Olivier Rascol, Wassilios G. Meissner. Current Concepts in the Treatment of Multiple System Atrophy. <i>Movement Disorders Clinical Practice</i>, 2015, 2, 1, 6–16.
Алгоритми за проследяване на заболяването
<u>Алгоритми за проследяване на заболяването:</u> МСА е бързо прогресиращо заболяване с приковаване към инвалидна количка, неразбираема реч, интермитентна уринарна кататеризация, инвалидизираща ортостатична хипотония и когнитивен дефицит (дизекзекутивен синдром). Прогнозата е лоша със средна преживяемост от 6-9 години. <u>Възможни усложнения:</u> Бронхопневмония (48%) и внезапна смърт (21%) са най-честите терминални състояния при МСА. Уринарната дисфункция при МСА често води до инфекции на долния уринарен тракт, като повече от 50% от пациентите с МСА страдат от рецидивиращи инфекции на долния уринарен тракт и значителен брой умират от усложнения.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. Papatsoris AG, Papapetropoulos S, Singer C, Deliveliotis C. Urinary and erectile dysfunction in multiple system atrophy (MSA). <i>Neurourol Urodyn.</i> 2008. 27(1):22-7. 2. Fanciulli A, Wenning GK. Multiple-system atrophy. <i>N Engl J Med.</i> 2015, 2;372(14):1375-6.
Алгоритми за рехабилитация на заболяването
<u>Алгоритми за рехабилитация на заболяването:</u> Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, както и съгласно международните критерии.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
<ol style="list-style-type: none"> 1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015. 3. Santiago Perez-Lloret, Olivier Flabeau, Pierre-Olivier Fernagut, Anne Pavy-Le Traon, María Verónica Rey, Alexandra Foubert-Samier, Francois Tison, Olivier

Rascol, Wassilios G. Meissner. Current Concepts in the Treatment of Multiple System Atrophy. Movement Disorders Clinical Practice, 2015, 2, 1, 6–16.

Необходими дейности за профилактика на заболяването (ако такива са приложими)

Дейности за профилактика на заболяването: Точната етиология на МСА-паркинсонизъм е все още неизвестна, но наличието на цитоплазмени агрегати от α -synuclein, предимно в олигодендроглия в комбинация с преобладаваща невродегенерация на стриатонигралните пътища са патологичен маркер на МСА-паркинсонов тип. Мутациите на COQ2 ген (4q21.23) (кодиращ ензима въввлечен в биосинтезата на коензим Q10) са наблюдавани множество фамилии с МСА, както и някои варианти на мутации, асоциирани с повишен риск от спорадичен МСА. Няма установени специфични профилактични мерки за заболяването.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Gilman, S., Wenning, G. K., Low, P. A., Brooks, D. J., Mathias, C. J., Trojanowski, J. Q., Wood, N. W., Colosimo, C., Durr, A., Fowler, C. J., Kaufmann, H., Klockgether, T., Lees, A., Poewe, W., Quinn, N., Revesz, T., Robertson, D., Sandroni, P., Seppi, K., Vidailhet, M. Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670-676, 2008.
2. Scholz, S. W., Houlden, H., Schulte, C., Sharma, M., Li, A., Berg, D., Melchers, A., Paudel, R., Gibbs, J. R., Simon-Sanchez, J., Paisan-Ruiz, C., Bras, J., and 26 others. SNCA variants are associated with increased risk for multiple system atrophy. Ann. Neurol. 65: 610-614, 2009. Note: Erratum: Ann. Neurol. 67: 277 only, 2010.

Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба

Създаването на Национален експертен център „Редки невродегенеративни заболявания, протичащи с когнитивни, поведенчески и моторни нарушения” за диагностика, лечение и проследяване и рехабилитация включително и на пациенти с това заболявания под ръководството на чл.кор.проф.д-р Л. Трайков, дмн (национален експерт с най-голям опит и принос за диагностиката и лечението на тези заболявания).

Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)

Опитът на кандидатстващия експертен център за диагноза и лечение на редки заболявания с атипичен паркинсонизъм, като мултисистемната атрофия датира от 2001 година със създаването на център за диагноза и лечение на невродегенеративни заболявания, протичащи с деменция и допълнително на център за диагноза и лечение на Паркинсонова болест. От дълги години този център е рефериран център за заболявания, протичащи с атипичен паркинсонизъм, като мултисистемната атрофия, особено за комплексни, редки и наследствени случаи. През годините вследствие на натрупания опит и труд, както и значителен брой на пациенти с тези редки заболявания, реферирани към нашите два центъра са осъществени няколко дисертации в областта: 1. Клинико-генетични корелации при невродегенеративни заболявания, протичащи с паркинсонизъм (защитена дисертация за доктор по медицина от д-р Радка Павлова, 2013 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Проучване на невропсихологичния профил при пациенти с Паркинсон плюс синдроми (защитена

дисертация за доктор по медицина от д-р Силвия Скелина, 2016 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. Клинико-генетични проучвания при фронтотемпорална деменция и сродни заболявания (защитена дисертация за доктор по медицински науки от д-р Шима Мехрабиан, 2016 г.). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, с атипичен паркинсонизъм, като мултисистемната атрофия с подробно фенотипизиране на всеки един случай, което дава възможност за добър мониторинг на пациентите, както и изследователски анализ върху характеристиката на отделните заболявания. Дейността на центъра по отношение на диагноза и лечение на редки заболявания, с атипичен паркинсонизъм, като мултисистемната атрофия, обхваща всички диагностични дейности съобразно новите диагностични критерии на тези заболявания, включително допълнителни изследвания, които са нужни за диференциална диагноза на атипични/ранни/наследствени случаи, включващи изследвания за биомаркери, невроизобразяващи и генетични фактори. Центъра е член на the European Multisystem Atrophy Study Group и International Parkinson and Movement Disorder Society.

Публикации:

1. Petrova M, Pavlova R, Zhelev Y, Mehrabian S, Raycheva M, Traykov L. Investigation of neuropsychological characteristics of very mild and mild dementia with Lewy bodies. *J Clin Exp Neuropsychol*. 2015 Dec 17:1-7.
2. Petrova M, Mehrabian-Spasova S, Aarsland D, Raycheva M, Traykov L. Clinical and Neuropsychological Differences between Mild Parkinson's Disease Dementia and Dementia with Lewy Bodies. *Dement Geriatr Cogn Dis Extra*. 2015 May 29;5(2):212-20.
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We report the results of a consensus conference on the diagnosis of multiple system atrophy (MSA). We describe the clinical features of the disease, which include four domains: autonomic failure/urinary dysfunction, parkinsonism and cerebellar ataxia, and corticospinal dysfunction. We set criteria to define the relative importance of these features. The diagnosis of possible MSA requires one criterion plus two features from separate domains. The diagnosis of probable MSA requires the criterion for autonomic failure/urinary dysfunction plus poor levodopa responsive parkinsonism or cerebellar ataxia. The diagnosis of definite MSA requires pathological confirmation.

Keywords: multiple system atrophy, parkinsonism, cerebellar ataxia, autonomic insufficiency, urinary dysfunction, glial cytoplasmic inclusions.

Consensus statement on the diagnosis of multiple system atrophy

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Multiple system atrophy (MSA), a progressive neurodegenerative disease of undetermined etiology, occurs sporadically and causes parkinsonism and cerebellar, autonomic, urinary, and pyramidal dysfunction in many combinations [1-4]. The disease affects both sexes, usually beginning in middle age and progressing over intervals of 1 to 18 years, with a median survival of 9.3 years from the first symptom [5,6]. The parkinsonian features include bradykinesia with rigidity, postural instability, hypokinetic speech, and often tremor, usually with a poor or unsustained response to chronic levodopa therapy. The cerebellar dysfunction consists of ataxia of gait, limb movements and speech, and disorders of extraocular movements [7]. Autonomic insufficiency results in orthostatic hypotension, often with an inadequate heart rate response to standing, male erectile dysfunction (MED), constipation, and decreased sweating [8,9]. Urinary symptoms include urgency, frequency, nocturia, incomplete bladder emptying, and incontinence [10]. The diagnosis of MSA requires primarily clinical assessment; however, a number of laboratory tests may help to support the diagnosis.

The neuropathological changes consist of a high density of glial cytoplasmic inclusions (GCIs) in association with degenerative changes in some or all of the following structures: putamen, caudate nucleus, globus pallidus, substantia nigra, locus ceruleus, inferior olives, pontine nuclei, cerebellar Purkinje cells, autonomic nuclei of the brainstem, and the intermediolateral cell columns and Onuf's nucleus in the spinal cord [11,12]. GCIs are ubiquitin-, tau- and α -synuclein-positive oligodendroglial inclusions [12].

Some efforts have been made to establish diagnostic criteria [4], but no consistent detailed guidelines have been developed. Accordingly, a consensus conference was convened on April 23 and 24, 1998 in Minneapolis, Minnesota, cosponsored by the American Autonomic Society and the American Academy of Neurology. The goal of the conference was to develop guidelines for the diagnosis of MSA. We achieved consensus on the items listed below and shown in Tables 1, 2, and 3. These guidelines have not yet been validated, and will almost certainly require further modification in the light of future experience.

Clinical domains

Autonomic and urinary dysfunction

Orthostatic hypotension (OH) may indicate autonomic failure and can be asymptomatic or symptomatic. When symptomatic, it typically occurs after the onset of MED and urinary symptoms. Symptoms of OH result from cerebral hypoperfusion, and syncope may occur. The consensus conference determined that the clinical diagnosis of probable MSA requires a reduction of systolic blood pressure by at least 30 mm Hg or of diastolic blood pressure by at least 15 mm Hg within 3 minutes of standing from the recumbent

* Please see "Conference Participants" section at the end of this article for a full listing of author affiliations.

Table 1. Clinical domains, features, and criteria used in the diagnosis of MSA*

I. Autonomic and urinary dysfunction
A) Autonomic and urinary features
1. Orthostatic hypotension (by 20 mm Hg systolic or 10 mm Hg diastolic)
2. Urinary incontinence or incomplete bladder emptying
B) Criterion for autonomic failure or urinary dysfunction in MSA
Orthostatic fall in blood pressure (by 30 mm Hg systolic or 15 mm Hg diastolic) or urinary incontinence (persistent, involuntary partial or total bladder emptying, accompanied by erectile dysfunction in men) or both
II. Parkinsonism
A) Parkinsonian features
1. Bradykinesia (slowness of voluntary movement with progressive reduction in speed and amplitude during repetitive actions)
2. Rigidity
3. Postural instability (not caused by primary visual, vestibular, cerebellar, or proprioceptive dysfunction)
4. Tremor (postural, resting, or both)
B) Criterion for parkinsonism in MSA
Bradykinesia plus at least one of items 2 to 4
III. Cerebellar dysfunction
A) Cerebellar features
1. Gait ataxia (wide based stance with steps of irregular length and direction)
2. Ataxic dysarthria
3. Limb ataxia
4. Sustained gaze-evoked nystagmus
B) Criterion for cerebellar dysfunction in MSA
Gait ataxia plus at least one of items 2 to 4
IV. Corticospinal tract dysfunction
A) Corticospinal tract features
1. Extensor plantar responses with hyperreflexia
B) Corticospinal tract dysfunction in MSA: no corticospinal tract features are used in defining the diagnosis of MSA

*A feature (A) is a characteristic of the disease and a criterion (B) is a defining feature or composite of features required for diagnosis.

position. Frequently this is accompanied by an inadequate increase in heart rate (less than 10 beats per minute). We note that this is a more pronounced degree of OH than established previously [4]. MED appears early and affects virtually all male patients with MSA, but the symptom has

Table 2. Diagnostic categories of MSA*

I. Possible MSA: One criterion plus two features from separate domains. When the criterion is parkinsonism, a poor levodopa response qualifies as one feature (hence only one additional feature is required).
II. Probable MSA: Criterion for: autonomic failure/urinary dysfunction plus poor levodopa-responsive parkinsonism or cerebellar dysfunction.
III. Definite MSA: Pathologically confirmed by the presence of a high density of glial cytoplasmic inclusions in association with a combination of degenerative changes in the nigrostriatal and olivopontocerebellar pathways.

*The features and criteria for each clinical domain are shown in Table 1.

Table 3. Exclusion criteria for the diagnosis of MSA

I. History
Symptomatic onset under 30 years of age
Family history of a similar disorder
Systemic diseases or other identifiable causes for features listed in Table 1
Hallucinations unrelated to medication
II. Physical examination
DSM criteria for dementia
Prominent slowing of vertical saccades or vertical supranuclear gaze palsy*
Evidence of focal cortical dysfunction such as aphasia, alien limb syndrome, and parietal dysfunction
III. Laboratory investigation
Metabolic, molecular genetic, and imaging evidence of an alternative cause of features listed in Table 1

*In practice, MSA is most frequently confused with Parkinson's disease or progressive supranuclear palsy (PSP) [25]. Mild limitation of upward gaze alone is nonspecific, whereas a prominent (>50%) limitation of upward gaze or any limitation of downward gaze suggests PSP. Before the onset of vertical gaze limitation, a clinically obvious slowing of voluntary vertical saccades is usually easily detectable in PSP and assists in the early differentiation of these two disorders [26].

low specificity. Urinary frequency, urgency, incontinence, or incomplete bladder emptying also occur early and commonly.

Parkinsonism

The majority of MSA patients develop parkinsonian features at some stage of the disorder. All these patients have bradykinesia; rigidity, postural instability, and tremor also often occur. The tremor is usually irregular and postural, often incorporating myoclonus. A classical pill-rolling parkinsonian rest tremor is uncommon. The parkinsonism in MSA can be asymmetric. The dysarthria is mainly hypokinetic, often mixed with other components [13]. The parkinsonian features usually respond poorly to chronic levodopa therapy; however, up to 30% of patients show a clinically significant response to levodopa therapy at some time in the course, but the response is usually sustained for less than five years [5,14,15]. These are the most challenging patients for accurate diagnosis.

Cerebellar dysfunction

Ataxia of gait, the most common cerebellar feature of MSA, often occurs accompanied by dysarthria, limb ataxia, and sometimes gaze-evoked nystagmus and ocular dysmetria. A common finding is saccadic pursuit movements. The dysarthria in patients with predominantly cerebellar dysfunction is mainly ataxic, often mixed with other components [13].

Corticospinal dysfunction

Extensor plantar responses with hyperreflexia occur in about 50% of MSA patients. Corticospinal signs can contribute to the diagnosis, but are less important than abnormalities in the other domains.

Response to levodopa

Levodopa responsiveness should be tested by administering escalating doses (with a peripheral decarboxylase inhibitor) over a 3-month period up to at least 1 g per day (if necessary and if tolerated). A positive response is defined as clinically significant improvement. This should be demonstrated by objective evidence such as an improvement of 30% or more on part III (motor examination) of the Unified Parkinson's Disease Rating Scale [16].

Laboratory investigations

Autonomic function tests, sphincter electromyography (EMG), and neuroimaging may be used to support the diagnosis, and neuroimaging is helpful in excluding other conditions. The abnormalities described below have been defined principally in clinically well-established cases rather than in the early stages of the disease. In the early stages, the tests may give equivocal results. We consider it premature to incorporate laboratory results into the entirely clinical guidelines that we established, but envision the future development of "Laboratory Supported" diagnostic categories.

Assessment of autonomic function can be assisted by a comprehensive battery that evaluates the distribution and severity of sudomotor, cardiovascular, and sympathetic adrenergic deficits [17,18]. Autonomic function tests may help separate MSA from Parkinson's disease and from idiopathic cerebellar degenerations [8].

Sphincter EMG can be useful in the diagnosis of MSA. Analysis of individual motor unit potentials recorded from the external anal sphincter usually shows changes indicating chronic reinnervation, with markedly prolonged motor units [10,19].

Magnetic resonance imaging (MRI) can assist the evaluation by detecting abnormalities of striatum, cerebellum, and brainstem, but can be normal in up to 20% of cases [20]. Striatal abnormalities may include putaminal atrophy, slit-like signal change at the posterolateral putaminal margin, and hypointensity of the putamen relative to the globus pallidus [21]. Infratentorial abnormalities include cerebellar and pontine atrophy, and signal change in the pons and middle cerebellar peduncles [22]. Studies are in progress to evaluate the utility of magnetic resonance spectroscopy, positron emission tomography, and single photon emission tomography.

Diagnostic categories

We established three diagnostic categories reflecting differing levels of certainty: definite, probable, and possible. The diagnosis of definite MSA can only be made after neuropathological examination of the central nervous system revealing the characteristic density and distribution of GCIs and degenerative changes outlined above. The diagnosis of probable or possible MSA can be made using different combinations of

clinical domains, criteria, and features, as indicated in Tables 1 and 2. Exclusion criteria are shown in Table 3.

Terminology

MSA is a distinct clinicopathological entity. The term should not be used to describe other neurodegenerative diseases affecting multiple systems. The use of confusing terms such as "multisystem degeneration" for MSA is inappropriate and now should be discouraged. We recommend designating patients as having MSA-P if parkinsonian features predominate or MSA-C if cerebellar features predominate [23,24]. These terms are intended to replace the striatonigral degeneration (SND) and sporadic olivopontocerebellar atrophy (sOPCA) types of MSA, respectively. The term Shy Drager syndrome has been widely misused, and is no longer useful.

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Second consensus statement on the diagnosis of multiple system atrophy



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ABSTRACT

Background: A consensus conference on multiple system atrophy (MSA) in 1998 established criteria for diagnosis that have been accepted widely. Since then, clinical, laboratory, neuropathologic, and imaging studies have advanced the field, requiring a fresh evaluation of diagnostic criteria. We held a second consensus conference in 2007 and present the results here.

Methods: Experts in the clinical, neuropathologic, and imaging aspects of MSA were invited to participate in a 2-day consensus conference. Participants were divided into five groups, consisting of specialists in the parkinsonian, cerebellar, autonomic, neuropathologic, and imaging aspects of the disorder. Each group independently wrote diagnostic criteria for its area of expertise in advance of the meeting. These criteria were discussed and reconciled during the meeting using consensus methodology.

Results: The new criteria retain the diagnostic categories of MSA with predominant parkinsonism and MSA with predominant cerebellar ataxia to designate the predominant motor features and also retain the designations of definite, probable, and possible MSA. Definite MSA requires neuropathologic demonstration of CNS α -synuclein-positive glial cytoplasmic inclusions with neurodegenerative changes in striatonigral or olivopontocerebellar structures. Probable MSA requires a sporadic, progressive adult-onset disorder including rigorously defined autonomic failure and poorly levodopa-responsive parkinsonism or cerebellar ataxia. Possible MSA requires a sporadic, progressive adult-onset disease including parkinsonism or cerebellar ataxia and at least one feature suggesting autonomic dysfunction plus one other feature that may be a clinical or a neuroimaging abnormality.

Conclusions: These new criteria have simplified the previous criteria, have incorporated current knowledge, and are expected to enhance future assessments of the disease.

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GLOSSARY

[¹¹C]HED = [¹¹C]hydroxyephedrine; AAN = American Academy of Neurology; AAS = American Autonomic Society; DSM-IV = *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition; DWI = diffusion-weighted imaging; ED = erectile dysfunction; FDG = [¹⁸F]fluorodeoxyglucose; FXTAS = fragile X-associated tremor/ataxia syndrome; MCP = middle cerebellar peduncle; MIBG = [¹²³I]metaiodobenzylguanidine; MR = magnetic resonance; MSA = multiple system atrophy; MSA-C = MSA with predominant cerebellar ataxia; MSA-P = MSA with predominant parkinsonism; OH = orthostatic hypotension; PD = Parkinson disease; SCA = spinocerebellar ataxia; SMC = Safety Monitoring Committee; RV = residual volume; UPDRS = Unified Parkinson's Disease Rating Scale.

Multiple system atrophy (MSA) is an adult-onset, sporadic, progressive neurodegenerative disease characterized by varying severity of parkinsonian features, cerebellar ataxia, autonomic failure, urogenital dysfunction, and corticospinal disorders.¹⁻⁴ The disease frequently begins with bladder dysfunction, and in males erectile dysfunction (ED) usually precedes this complaint.⁵ The presenting motor disorder most commonly consists of parkinsonism with bradykinesia, rigidity, gait instability, and at times tremor, but cerebellar ataxia is the initial motor disorder in a substantial percentage of patients.² The defining neuropathology of MSA consists of degeneration of striatonigral and olivopontocerebellar structures accompanied by profuse

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numbers of distinctive glial cytoplasmic inclusions formed by fibrillized α -synuclein proteins.⁶⁻⁸ Although the etiology is unknown, this disorder, like Parkinson disease (PD) and dementia with Lewy bodies, seems to result from a disturbance of α -synuclein and is designated as an α -synucleinopathy.

A consensus conference on diagnosis held in 1998 defined two categories, MSA with predominant parkinsonism (MSA-P) and MSA with predominant cerebellar ataxia (MSA-C).³ Three levels of certainty were established, possible, probable, and definite MSA, with the diagnosis of definite MSA requiring autopsy confirmation. These guidelines emphasized the importance of autonomic by requiring this feature for the diagnosis of probable MSA. Validation studies of the consensus criteria demonstrated high predictive accuracy but suboptimal sensitivity, particularly in the early stages of the disease.^{9,10} For the category of possible MSA, predictive accuracy was relatively lower at the first neurologic visit, but sensitivity was higher at this time point as compared with criteria defining probable MSA.

Widely accepted, the original consensus criteria have served as the gold standard for diagnosis. Nevertheless, the criteria used separate features and criteria for diagnosis that were complex and difficult to keep in mind. Moreover, additional information relevant to diagnostic criteria has accumulated since these criteria were published, including clinical and laboratory studies,⁴ neuropathologic and biochemical findings,^{8,11} and neuroimaging studies.¹²⁻¹⁴ Accordingly, the time had come to initiate a second consensus conference to develop new guidelines for diagnosis. These guidelines are intended for both practicing clinical neurologists and investigators studying the disease.

METHODS Development of this consensus conference began with a grant application to the NIH for support. The grant was funded, additional support was obtained, and the American Academy of Neurology (AAN) agreed to cosponsor the event. A Steering Committee was selected that included investigators with expertise in MSA-P (G.K.W.), MSA-C (S.G.), and autonomic failure (P.A.L.). The Steering Committee selected members for the consensus group based on the members' expertise in the parkinsonian, cerebellar, and autonomic features; in the neu-

ropathologic and biochemical disturbances; and in the structural and functional imaging characteristics of the disease. Selection of members required evidence that they were active investigators and published in the areas relevant to the diagnostic considerations, and included efforts to involve qualified women and minorities in the group. All of those who were contacted initially agreed to serve, and they were divided into five task groups: MSA-P, MSA-C, Autonomic, Neuropathology, and Neuroimaging. A Chair was appointed for each group based on a long track record of leadership in the discipline of the group. The MSA-P group included Gregor K. Wenning (Chair), Carlo Colosimo, Andrew Lees, Werner Poewe, Niall Quinn, and Marie Vidailhet. Members of the MSA-C group were Nicholas W. Wood (Chair), Alexandra Dürr, Thomas Klockgether, and Sid Gilman. The Autonomic group consisted of Christopher J. Mathias (Chair), Clare J. Fowler, Horacio Kaufmann, Phillip A. Low, David Robertson, and Paola Sandroni. The Neuroimaging group included David J. Brooks (Chair) and Klaus Seppi. The Neuropathology group consisted of John Q. Trojanowski (Chair) and Tamas Revesz.

Each task group received from the Steering Committee a request to develop a position paper regarding consensus criteria for diagnosis limited to their area of expertise. The task group chairs initially wrote the position papers, sent them to group members for comments and criticism, and after an iterative process, sent their draft reports to the Steering Committee. The Committee reviewed them and returned them to the task group chairs with comments as needed. Final drafts of the position papers were circulated to the entire membership of the consensus committee in advance of the meeting. A 2-day meeting was held in Boston, Massachusetts, on April 26 and 27, 2007, immediately preceding the AAN meeting. The meeting involved the use of consensus methodology to arrive at the current criteria for diagnosis. Consensus methodology uses the collected judgment of seasoned investigators closely familiar with the disease from years of experience. The consensus process involves the following principles: all members 1) contribute to the discussion, 2) can state each issue in their own words, 3) have the opportunity and time to express their opinion about each issue, and 4) agree to take responsibility for the implementation of a decision. Members who disagree will agree to support the group decision initially on a trial basis, pending further discussion. Achieving consensus requires that all members 1) listen nonjudgmentally to the opinions of other members and 2) check for understanding by summarizing what they think they hear while building on each other's thoughts and exploring minority opinions. The advantages of this methodology are that the quality of a decision is often excellent because it is based on shared information and opinion; and the level of support for each decision is often great because all members participate in making the decision and there is no minority group whose opinions are discounted. The Steering Committee developed the present article with contributions from the entire membership of the consensus group.

RESULTS Diagnostic categories. Similar to the first consensus conference, we determined that the diagnosis of MSA should be divided into three groups. The first, definite MSA, requires the neuropathologic findings of widespread and abundant CNS α -synuclein-positive glial cytoplasmic inclusions (Papp-Lantos inclusions) in association with neurodegenerative changes in striatonigral or olivopontocerebellar

Table 1 Criteria for the diagnosis of probable MSA

A sporadic, progressive, adult (>30 y)-onset disease characterized by

- Autonomic failure involving urinary incontinence (inability to control the release of urine from the bladder, with erectile dysfunction in males) or an orthostatic decrease of blood pressure within 3 min of standing by at least 30 mm Hg systolic or 15 mm Hg diastolic and
- Poorly levodopa-responsive parkinsonism (bradykinesia with rigidity, tremor, or postural instability) or
- A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction)

MSA = multiple system atrophy.

structures.¹⁵ Criteria for probable MSA are listed in table 1 and for possible MSA are listed in tables 2 and 3.³ Patients with predominantly parkinsonian features should continue to be designated MSA-P, and patients with predominantly cerebellar ataxia should be designated MSA-C. We appreciate that the predominant motor feature can change with time; thus, patients who present with cerebellar ataxia can develop increasingly severe parkinsonian features until these latter features dominate the clinical presentation. Hence the designation of MSA-P or MSA-C refers to the predominant feature at the time the patient is evaluated, and the predominant feature can change with time. Also, determining the predominant feature becomes a matter of clinical judgment in subjects with combinations of parkinsonian and cerebellar features. We do not recommend using the term "MSA-mixed" to describe patients with combinations of cerebellar ataxia and parkinsonian features, because this category could be used as a default for any combination of ataxia and parkinsonism, irrespective of the severity of each. Disease onset is defined as the initial presentation of any motor problem, whether parkinsonian or cerebellar, or autonomic features, as defined in the criteria for possible MSA, with the exception of male ED. Although the disease process must start earlier within the CNS, for research purposes, a pragmatic definition of disease onset is required. We excluded ED in men⁵ and reduced genital sensitivity in women¹⁶ because these symptoms have myriad causes in older people. Table 1 provides the criteria for autonomic failure, which is an integral part of MSA, and table 4 gives features supporting and not supporting a diagnosis of MSA for cases with possible MSA.

Autonomic failure. Orthostatic hypotension (OH) may indicate autonomic failure and can be asymptomatic or symptomatic. When symptomatic, it frequently occurs after the onset of ED and urinary symptoms.⁵ Symptoms of OH result from hypoperfusion, and syncope may occur.¹⁷ The clinical diagnosis of probable MSA requires a reduction of systolic blood pressure by at least 30 mm Hg or of diastolic blood pressure by at least 15 mm Hg after 3 minutes of standing from a previous 3-minute interval in the recumbent position. This orthostatic decline is usually accompanied by a compensatory increase in heart rate that is inadequately low for the level of blood pressure decline. We note that this is a more pronounced decrease of blood pressure than recommended previously in the American Autonomic Society (AAS)-AAN consensus statement on the definition of orthostatic hypotension.¹⁸ Blood pressure can be decreased additionally by drugs, fluid depletion, food ingestion, an increased temperature, and physical deconditioning. Other disorders known to cause OH, such as diabetes mellitus with autonomic neuropathy, should be excluded or at least taken into account.

Genitourinary dysfunction. ED is often the earliest symptom of MSA and affects virtually all male patients⁵; apart from one report of decreased genital sensitivity,¹⁶ there is little information about female sexual dysfunction in MSA. Because the prevalence of ED increases with age, the symptom has a low specificity; however, preserved erectile function makes a diagnosis of MSA unlikely. Urinary complaints are common in the aging population, but the recent, unexplained onset of urinary incontinence,

Table 2 Criteria for possible MSA

A sporadic, progressive, adult (>30 y)-onset disease characterized by

- Parkinsonism (bradykinesia with rigidity, tremor, or postural instability) or
- A cerebellar syndrome (gait ataxia with cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction) and
- At least one feature suggesting autonomic dysfunction (otherwise unexplained urinary urgency, frequency or incomplete bladder emptying, erectile dysfunction in males, or significant orthostatic blood pressure decline that does not meet the level required in probable MSA) and
- At least one of the additional features shown in table 3

MSA = multiple system atrophy.

Table 3 Additional features of possible MSA

Possible MSA-P or MSA-C

- Babinski sign with hyperreflexia
- Stridor

Possible MSA-P

- Rapidly progressive parkinsonism
- Poor response to levodopa
- Postural instability within 3 y of motor onset
- Gait ataxia, cerebellar dysarthria, limb ataxia, or cerebellar oculomotor dysfunction
- Dysphagia within 5 y of motor onset
- Atrophy on MRI of putamen, middle cerebellar peduncle, pons, or cerebellum
- Hypometabolism on FDG-PET in putamen, brainstem, or cerebellum

Possible MSA-C

- Parkinsonism (bradykinesia and rigidity)
- Atrophy on MRI of putamen, middle cerebellar peduncle, or pons
- Hypometabolism on FDG-PET in putamen
- Presynaptic nigrostriatal dopaminergic denervation on SPECT or PET

MSA = multiple system atrophy; MSA-P = MSA with predominant parkinsonism; MSA-C = MSA with predominant cerebellar ataxia; FDG = [¹⁸F]fluorodeoxyglucose.

especially in men, and incomplete bladder emptying increase the likelihood of a diagnosis of MSA. Constipation often accompanies the other autonomic symptoms.

Laboratory investigations of autonomic failure. Autonomic failure can be evaluated not only by a history of urinary incontinence and orthostatic blood pressure measurements in the clinic, but also by a comprehensive battery that examines the distribution and severity of cardiovascular, sudomotor, and urinary bladder deficits.¹⁹ Cardiovascular and sudomotor autonomic function tests may help to separate MSA

Table 4 Features supporting (red flags) and not supporting a diagnosis of MSA

Supporting features	Nonsupporting features
• Orofacial dystonia	• Classic pill-rolling rest tremor
• Disproportionate antecollis	• Clinically significant neuropathy
• Camptocormia (severe anterior flexion of the spine) and/or Pisa syndrome (severe lateral flexion of the spine)	• Hallucinations not induced by drugs
• Contractures of hands or feet	• Onset after age 75 y
• Inspiratory sighs	• Family history of ataxia or parkinsonism
• Severe dysphonia	• Dementia (on DSM-IV)
• Severe dysarthria	• White matter lesions suggesting multiple sclerosis
• New or increased snoring	
• Cold hands and feet	
• Pathologic laughter or crying	
• Jerky, myoclonic postural/action tremor	

MSA = multiple system atrophy; DSM-IV = *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition.

from other sporadic cerebellar ataxias and from PD.²⁰ Measurement of urine residual volume (RV) by ultrasound can reveal incomplete bladder emptying of >100 mL. RV tends to increase as MSA progresses. Imaging of cardiac innervation with SPECT and [¹²³I]metaiodobenzylguanidine (MIBG) and with PET and [¹⁸F]fluorodopa in many reports have shown preserved sympathetic postganglionic neurons in MSA, in contrast to PD²¹; however, some MIBG studies have shown denervation in MSA,²² and a recent investigation with PET and [¹¹C]hydroxyephedrine ([¹¹C]HED) revealed severe cardiac denervation in MSA.²³

Parkinsonism. Most MSA patients develop parkinsonism (bradykinesia with rigidity, tremor, or postural instability) at some stage. The tremor is usually irregular and postural/action, often incorporating myoclonus, but a classic pill-rolling rest tremor is uncommon. The parkinsonism can be asymmetric. Postural instability, as defined by item 30 of the Unified Parkinson's Disease Rating Scale (UPDRS) part III (motor examination),²⁴ occurs earlier and progresses more rapidly than in PD. Moreover, the UPDRS part III score typically worsens by less than 10% per annum in PD but by more than 20% in MSA.²⁵

Levodopa responsiveness. Parkinsonism usually responds poorly to chronic levodopa therapy; however, up to 30% of patients show a clinically significant, but usually waning, response.²⁶ Responsiveness should be tested with escalating doses of levodopa with a peripheral decarboxylase inhibitor over 3 months up to at least 1 g/d (if necessary and tolerated). A positive response is defined as clinically significant motor improvement. This should be demonstrated by objective evidence such as an improvement of 30% or more on part III of the UPDRS or on part II of the Unified Multiple System Atrophy Rating Scale.²⁷

Sleep disorders. REM sleep behavior disorder and obstructive sleep apnea occur frequently in MSA, but also affect PD patients and are not diagnostically definitive.²⁸

Laboratory investigations. Structural and functional imaging can assist diagnosis. MRI demonstration of putaminal, pontine, and middle cerebellar peduncle (MCP) atrophy is helpful in both MSA-P and MSA-C.²⁹ T2-signal changes on 1.5-tesla MRI in the basal ganglia and brainstem can be helpful, including posterior putaminal hypointensity, hyperintense lateral putaminal rim, hot cross bun sign, and MCP hyperintensities.

Functional imaging demonstration of striatal or brainstem hypometabolism by PET with [¹⁸F]fluorodeoxyglucose can help in the diagnosis of MSA.¹⁴ In the absence of clinically evident ataxia in a patient

with parkinsonian features, demonstration of cerebellar hypometabolism can point to the diagnosis of MSA-P rather than PD. Conversely, in the absence of parkinsonian features in a patient with cerebellar ataxia, evidence of nigrostriatal dopaminergic denervation from functional imaging (SPECT and PET) may point to the diagnosis of MSA-C.³⁰ As noted above, cardiac sympathetic postganglionic imaging with SPECT shows denervation in PD³¹ and uncommonly in MSA,²² but PET with [¹¹C]HED may reveal extensive denervation in MSA.²³

Other imaging techniques, such as brain parenchymal sonography, magnetic resonance (MR) spectroscopy, MR diffusion-weighted imaging (DWI), MR diffusion tensor imaging, MR magnetization transfer imaging, and MR voxel-based morphometry remain investigational; however, MR DWI has been shown to discriminate MSA-P, even in the early disease stages, from PD and from healthy controls on the basis of increased putaminal and MCP diffusivity measures.³¹

The CSF neurofilament light chain and tau tests have been reported to differentiate MSA from PD but remain in an exploratory phase of development.³²

Cerebellar ataxia. Ataxia of gait, the most common cerebellar feature of MSA-C, is often accompanied by ataxia of speech (cerebellar dysarthria) and cerebellar oculomotor dysfunction. Limb ataxia may be seen but is generally less prominent than gait or speech disturbances. Although gaze-evoked nystagmus occurs in the majority of later-stage MSA-C patients, earlier oculomotor abnormalities may not involve nystagmus, but include square wave jerks, jerky pursuit, and dysmetric saccades. Limitations of supranuclear gaze and severe slowing of saccadic velocities are not features of MSA.

Differential diagnosis of adult-onset cerebellar ataxia.

The criteria for probable MSA-C shown in table 1 have high predictive value.^{9,10} MSA-C generally presents clinically as a midline cerebellar disorder that progresses more rapidly than other late-onset sporadic ataxias; typically a patient becomes wheelchair dependent by 5 years after onset.³³ The features detailed in tables 2 and 3 for possible MSA help to provide strong indicators of the diagnosis of MSA-C, and table 4 indicates features supporting and not supporting a diagnosis of MSA. Clinicians evaluating patients with progressive ataxia should include a large differential diagnostic list, because many diseases can produce an adult-onset progressive ataxia. The dominantly inherited spinocerebellar ataxias (SCAs) can result in an apparently sporadic disorder, because even with a negative family history, there is a 15% to 20% chance of a mutation in one of the polyglutamine SCAs, notably SCAs 1, 2, 3, 6, and 7. Fragile

X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder with core features of action tremor and ataxia of gait.³⁴ Frequently this disorder includes parkinsonism, abnormalities of executive function, dementia, neuropathy, and autonomic failure. FXTAS results from a premutation (moderate expansions of 55–200 repeats) of a CCG trinucleotide in the fragile X mental retardation 1 gene, the gene that causes fragile X syndrome when the full mutation develops (over 200 CCG repeats). A search for the fragile X mutation in a large series of possible, probable, and pathologically confirmed cases suggests the frequency is less than 1%.³⁵ FXTAS shows characteristic MRI features, hyperintensity in the middle cerebellar peduncles and supratentorial white matter, that may overlap with those of MSA. A diagnosis of paraneoplastic disease should be considered in patients with an aggressive clinical course with or without general systemic malaise, and a search for appropriate antibodies and for the putative primary should be undertaken. After these diseases have been excluded, and anatomic imaging shows cerebellar and brainstem atrophy, patients usually receive the diagnosis of sporadic adult-onset ataxia, which is also known as idiopathic late-onset cerebellar ataxia or sporadic olivopontocerebellar atrophy.³⁶ The cause of this disorder is unknown, and many patients who develop this disorder do not progress to MSA.

Findings that cast doubt on the diagnosis of MSA-C.

The presence of a family history of a similar disorder makes the diagnosis of MSA-C unlikely; one of the SCAs should be considered. Nevertheless, recent studies of several multiplex families suggest familial MSA may be due to autosomal recessive inheritance,³⁷ and autopsy-confirmed MSA has been reported in association with an abnormal expansion of one allele of the SCA type 3 gene.³⁸ Dementia also makes the diagnosis of MSA-C unlikely. MRI studies showing supratentorial white matter lesions other than those commonly seen in this age group make the diagnosis doubtful and raise the possibility of MS.

Differences between the first and second consensus criteria.

Although the current criteria have the same structure as the earlier criteria, there are differences in each category. In the previous criteria for definite MSA, α -synuclein-positive glial cytoplasmic inclusions were not required. The previous criteria for probable MSA used separate "features" and "criteria," which have been abandoned in the current criteria. Similarly, the separate "features" and "criteria" in possible MSA have been deleted and, in addition to parkinsonism or a cerebellar syndrome, now there must be one feature involving autonomic dysfunction.

tion plus one other finding, and the latter is detected either with clinical examination or with imaging.

DISCUSSION On clinical presentation, MSA appears with a combination of autonomic failure with parkinsonism or cerebellar ataxia or both, and the previous criteria and the present criteria recognize this fundamental feature of the disease. The current criteria also retain the distinctions between levels of diagnostic certainty, using the term *definite MSA* for subjects with autopsy demonstration of typical histologic features,¹⁵ *probable MSA* for patients with autonomic failure plus parkinsonism or cerebellar ataxia, and *possible MSA* for people with clinical findings that do not as yet clearly represent this disease.

The principal differences between the current criteria and the previous criteria concern clinical domains and requirements for the diagnosis of possible MSA. In the previous criteria, we used "features" to describe clinical findings and "criteria" to indicate the features that could be used for diagnosis. This distinction proved to be confusing and difficult to retain; hence it has been discarded. The current criteria include straightforward descriptions of the clinical findings required for the diagnoses of probable and possible MSA. The diagnosis of probable MSA is now considerably simplified, as shown in table 1. The diagnosis of possible MSA has been changed to require at least one feature suggesting autonomic dysfunction in addition to parkinsonism or a cerebellar syndrome. At least one additional feature will be required for this diagnosis and can include findings on history, clinical examination, and results from either structural or functional imaging. This change, particularly the requirement of a feature suggesting autonomic dysfunction, hopefully will decrease the false positives that characterize clinical diagnosis in the early stages of the disorder.

The new criteria were created using consensus methodology, as were the first consensus criteria. This methodology presents the advantage of using the collected experience of active investigators, but the disadvantage that the criteria do not result from an evidence-based approach. We anticipate that validation studies will be performed in the future and that these studies will show the high predictive accuracy of the first set of criteria and hopefully better sensitivity in the early stages of the disease than the previous criteria.^{9,10}

We anticipate that the new criteria will be less cumbersome to apply in patients with possible MSA than the first set of criteria. The principal differences between the first and second set of consensus criteria are in the group with possible MSA, and in this group it is possible now to use both clinical and im-

aging results to buttress the diagnosis in subjects with parkinsonian features or cerebellar dysfunction plus autonomic symptoms that do not meet the level needed for the diagnosis of probable MSA. In the latter group, we use criteria for autonomic failure that are more rigorous than those used by the AAS and AAN.¹⁸ This is to ensure a high level of accuracy in the diagnosis of MSA, because the disease is a grave one and carries the prognosis of a markedly shortened life span.

AFFILIATIONS

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DISCLOSURE

S.G. serves as Chair of the Safety Monitoring Committee (SMC) for the Elan and Wyeth trials of immunotherapy for Alzheimer disease and of the SMC for the Elan and Transition trials of scyllo-inositol for Alzheimer disease. He has served as a consultant for Wyeth, ReNeuron, Adamas, and Kyowa and has grant support from Cortex Pharmaceuticals. He is a consultant for PPD Development, Longitude Capital, and the Gerson Lehrman Group and a member of the Board of Directors of Balboa Bioscience. C.C. has received grant support and other honoraria from Allergan, Boehringer-Ingelheim, Eli-Lilly, GlaxoSmithKline, and Ipsen. W.P. has served as a consultant for GSK, Novartis, Teva, Boehringer Ingelheim, Schwarz Pharma/UCB, AstraZeneca, and General Electric. N.Q. has served as a consultant for Medtronic, Schwarz-Pharma, UCB, Boehringer & Ingelheim, GlaxoSmithKline, Dainippon Sumitomo, Solvay, Teva, and Novartis. The remaining authors have no disclosures.

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Multiplex Families With Multiple System Atrophy

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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 isoform; PP2R2B), SCA17 (TATA box binding protein, TBP) and DRPLA (atrophia 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.

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MULTIPLE SYSTEM ATROPHY (MSA) is a sporadic neurodegenerative disorder characterized by various combinations of parkinsonism, cerebellar ataxia, and autonomic failure.¹ 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.²⁻⁴ 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.⁵ 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.^{6,7} 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.¹ 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- μ m 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-A β compo-

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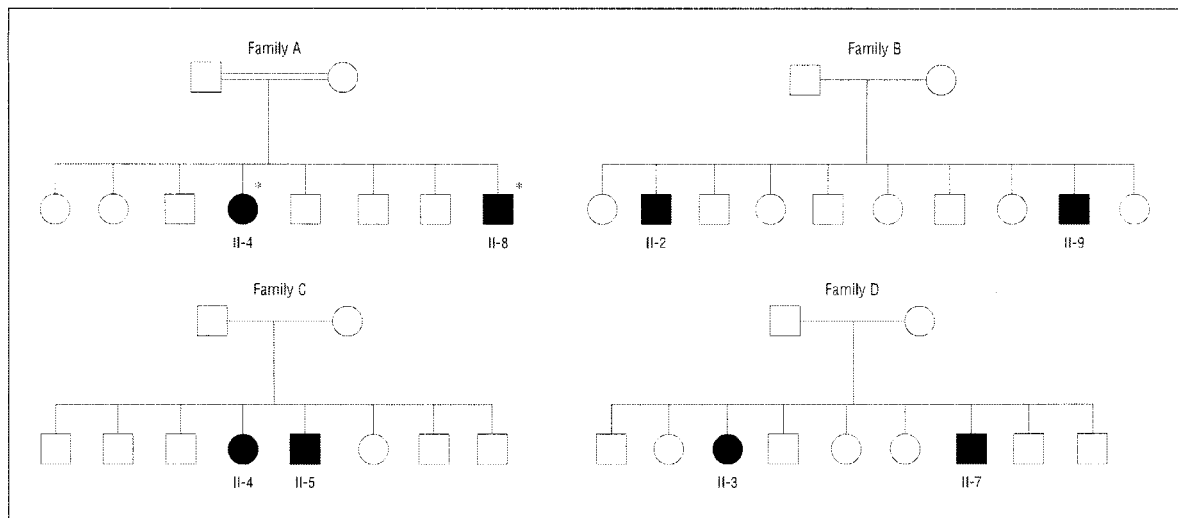


Figure 1. Four multiplex families with multiple system atrophy. The affected siblings in family A were born from a consanguineous marriage. In family A, both affected patients (II-4 and II-8) had retinitis pigmentosa (denoted by an asterisk) but not the other siblings. Squares represent men; circles, women; affected individuals, solid symbols; unaffected individuals; open symbols.

ment 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; *ATXN1* [for gene nomenclature see <http://www.gene.ucl.ac.uk/nomenclature>]⁹),¹⁰ SCA2 (*ATXN2*),¹¹⁻¹³ Machado-Joseph/SCA3 (*ATXN1*),¹⁴ SCA6 (*ATXN1*),¹⁵ SCA7 (*ATXN7*),¹⁶ SCA12 (protein phosphatase 2, regulatory subunit B, β isoform, *PP2R2B*),¹⁷ SCA17 (TATA box binding protein, *TBP*),^{18,19} and dentatorubral-pallidoluysian atrophy (DRPLA [atrophin 1, *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 (*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 pos-

terolateral 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 (*PPP2R2B*), SCA17 (*TBP*), and DRPLA (*ATN1*) gave normal results. No mutations in the *SNCA* gene were found in the family members.

REPORT OF CASES

FAMILY A

Patient II-4

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

Table. Clinical Findings Among 4 Multiplex Families With Multiple System Atrophy (MSA)

Finding	Family and Affected Individuals							
	A		B		C		D	
	II-4	II-8	II-2	II-9	II-4	II-5	II-3	II-7
Sex	Female	Male	Male	Male	Female	Male	Female	Male
Age at onset, y	68	62	72	63	68	67	69	58
Age at examination, y	71	66	73	66	72	68	72	63
Initial symptoms	Tremor	Ataxia	Tremor	Tremor	Tremor	Ataxia	Akinesia	Impotence
Parkinsonism	*	*	*	*	†	†	*	...
Cerebellar sign	‡	‡	...	‡	‡	‡	...	‡
Muscle stretch reflexes								
Upper limb	Enhanced	Normal	Normal	Normal	Normal	Normal	Enhanced	Enhanced
Lower limb	Enhanced	Normal	Normal	Normal	Normal	Normal	Enhanced	Enhanced
Babinski sign	‡	‡
Urinary dysfunction	‡	‡	‡	‡	...	‡	‡	‡
Orthostatic hypotension	‡	‡	‡
Response to levodopa	Poor	Poor	Poor	Poor	Poor	Poor	Poor	Poor
Brain magnetic resonance imaging								
Cerebellar atrophy	NE	‡	...	†	...	‡	‡	‡
Pontine atrophy	NE	‡	...	‡	...	*	‡	*
Cross sign	NE	‡	...	‡	...	‡	...	‡
Slitlike signal change at the posterolateral putaminal margin	NE	‡	‡	‡	‡	‡
Criteria by Gilman et al ¹	Definite	Probable	Probable	Probable	Possible	Probable	Possible	Probable
Phenotypes	MSA-P	MSA-P	MSA-P	MSA-P	MSA-P+C	MSA-P+C	MSA-P	MSA-C
Complication	RP	RP	Rheumatoid arthritis

Abbreviations: MSA-C, MSA of the cerebellar type; MSA-P, MSA with predominant parkinsonism; NE, not examined; RP, retinitis pigmentosa; ellipsis, not applicable.

*Severe finding.

†Moderate finding.

‡Finding present.

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 α -synuclein-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-dopadecarboxylase inhibitor. He had difficulty in urination at age 63 years, orthostatic hypotension at age 64 years, and truncal ataxia at age

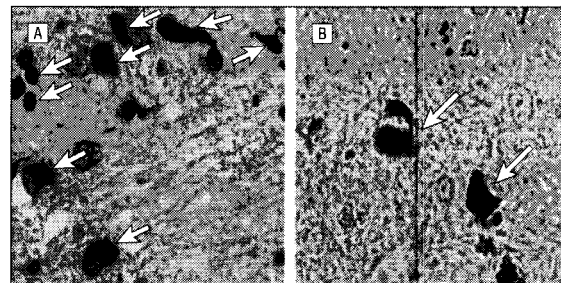


Figure 2. Histopathologic features of the pons from patient II-4 in family A. A, Argyrophilic glial cytoplasmic inclusions (arrows) (Gallyas-Braak stain). B, Abnormal accumulation of α -synuclein-precursor of the non-A β component of Alzheimer disease amyloid (NACP) in glial cells (arrows).

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 B₁, B₁₂, and E levels were normal. Nerve conduction studies revealed no abnormalities. Brain MR imaging showed mild atrophy of the

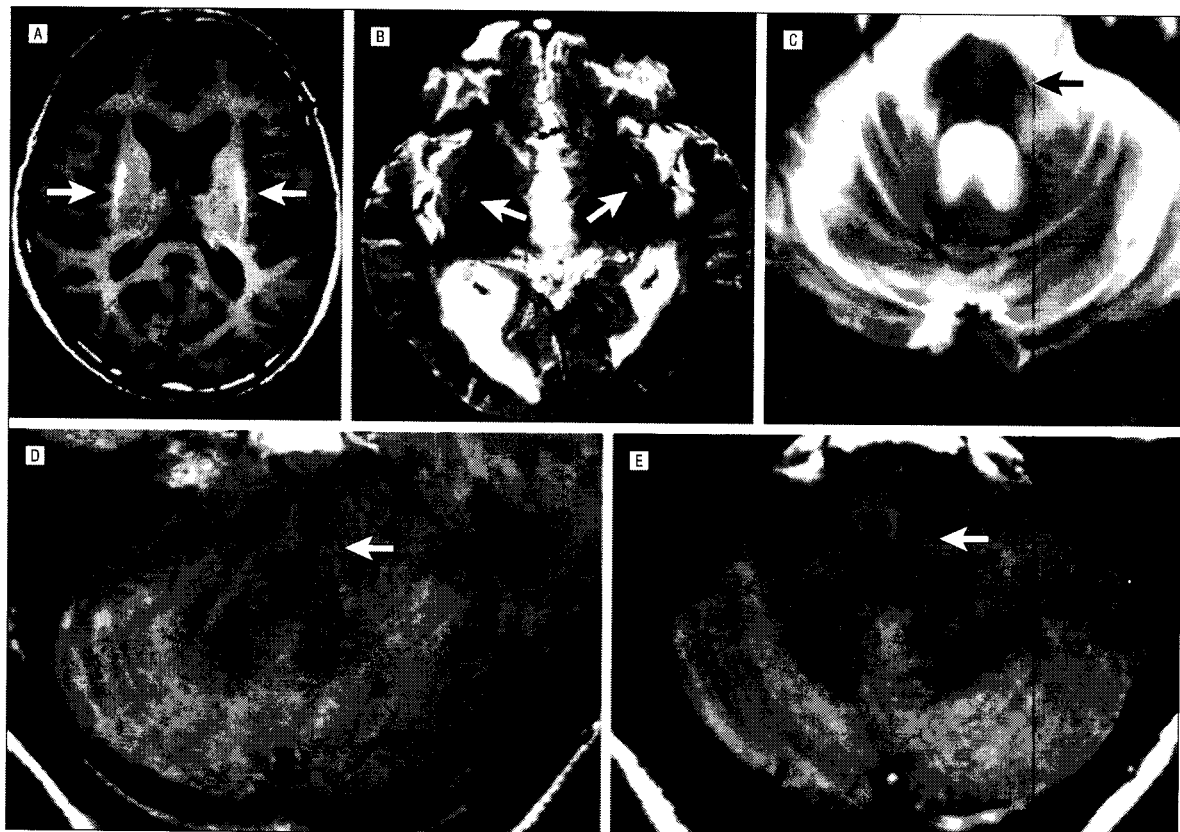


Figure 3. Representative brain magnetic resonance images of patients in the 4 families. A, T1-weighted image of patient II-8 in family A showing slitlike signal change at the posterolateral putaminal margin (arrows). B, T2-weighted image of patient II-2 in family B showing slitlike signal change at the posterolateral putaminal margin (arrows). C, T2-weighted image of patient II-9 in family B showing atrophy of the cerebellar vermis and pons with cross sign (arrow). D, Proton density-weighted image of patient II-5 in family C showing cross sign in the pons (arrow). E, Proton density-weighted image of patient II-7 in family D showing cross sign in the pons (arrow).

pons and cerebral cortex on T1-weighted imaging. Atrophy of the pontine base accompanied by cross sign²² on T2-weighted and proton density-weighted imaging was observed. Slitlike signal changes with hyperintensity on T1-weighted imaging and hypointensity on T2-weighted imaging²³ 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 mar-

gin 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–dopadecarboxylase 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 al¹ 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 palsy²³ or corticobasal degeneration.²³ 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,^{24,25} although such findings have been described infrequently in other diseases, including SCA2,²⁶ Machado-Joseph disease,²⁷ progressive supranuclear palsy,²⁸ and corticobasal degeneration.²⁸ 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 al²⁹ 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 al³⁰ 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.³¹ 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.^{6,7} 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 ($\chi^2=1.53, P=.22$).³² 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,³³ 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.³⁴ Second, the clinical phenotypes were concordant between 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).^{35,36} 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,³⁰ 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,^{2,4} 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.³⁷ 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.

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ORIGINAL ARTICLE

Mutations in *COQ2* in Familial and Sporadic Multiple-System Atrophy

The Multiple-System Atrophy Research Collaboration

 ABSTRACT

BACKGROUND

Multiple-system atrophy is an intractable neurodegenerative disease characterized by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction. Although multiple-system atrophy is widely considered to be a nongenetic disorder, we previously identified multiplex families with this disease, which indicates the involvement of genetic components.

METHODS

In combination with linkage analysis, we performed whole-genome sequencing of a sample obtained from a member of a multiplex family in whom multiple-system atrophy had been diagnosed on autopsy. We also performed mutational analysis of samples from members of five other multiplex families and from a Japanese series (363 patients and two sets of controls, one of 520 persons and one of 2383 persons), a European series (223 patients and 315 controls), and a North American series (172 patients and 294 controls). On the basis of these analyses, we used a yeast complementation assay and measured enzyme activity of parahydroxybenzoate-polyprenyl transferase. This enzyme is encoded by the gene *COQ2* and is essential for the biosynthesis of coenzyme Q₁₀. Levels of coenzyme Q₁₀ in lymphoblastoid cells and brain tissue were measured on high-performance liquid chromatography.

RESULTS

We identified a homozygous mutation (M128V-V393A/M128V-V393A) and compound heterozygous mutations (R387X/V393A) in *COQ2* in two multiplex families. Furthermore, we found that a common variant (V393A) and multiple rare variants in *COQ2*, all of which are functionally impaired, are associated with sporadic multiple-system atrophy. The V393A variant was exclusively observed in the Japanese population.

CONCLUSIONS

Functionally impaired variants of *COQ2* were associated with an increased risk of multiple-system atrophy in multiplex families and patients with sporadic disease, providing evidence of a role of impaired *COQ2* activities in the pathogenesis of this disease. (Funded by the Japan Society for the Promotion of Science and others.)

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MULTIPLE-SYSTEM ATROPHY IS A PROGRESSIVE neurodegenerative disease that is clinically characterized by autonomic failure in addition to various combinations of parkinsonism, cerebellar ataxia, and pyramidal dysfunction. The term multiple-system atrophy was introduced in 1969 to encompass the disease entities of olivopontocerebellar ataxia, striatonigral degeneration, and the Shy-Drager syndrome, on the basis of neuropathological findings in these disorders.¹ Multiple-system atrophy is characterized by the development of cytoplasmic aggregates of α -synuclein, primarily in oligodendroglia.²⁻⁷ However, the pathogenic mechanisms underlying this disease remain unknown, making it difficult to develop effective therapies.

The disorder is classified into two subtypes: subtype C, characterized predominantly by cerebellar ataxia, and subtype P, characterized predominantly by parkinsonism.⁸ Among patients with multiple-system atrophy, subtype C has been reported to be more prevalent than subtype P in the Japanese population (65 to 67% vs. 33 to 35%),^{9,10} whereas subtype P has been reported to be more prevalent than subtype C in Europe (63% vs. 34%)¹¹ and North America (60% vs. 13%, with 27% of cases unclassified).¹² Although multiple-system atrophy has been defined as a non-genetic disorder until recently, several multiplex families with the disease have been described, indicating that strong genetic factors confer susceptibility to the disease.¹³⁻¹⁵

METHODS

PATIENTS AND MULTIPLEX FAMILIES

Patients with multiple-system atrophy were enrolled in the study on the basis of research protocols that were approved by the institutional review board at each participating center. Written informed consent was obtained from all participants.

The diagnosis of multiple-system atrophy was made on the basis of the current consensus criteria for the disease.⁸ Four Japanese families (Families 1 through 4, whose members have been described previously¹³) and two additional Japanese families (Family 8 and Family 12) were enrolled in this study (Fig. 1). In Family 1, the parents were first-degree cousins, which is consistent with autosomal recessive inheritance. The clinical features of these families are sum-

marized in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Autopsy findings for Participants II-4¹³ and II-8 in Family 1 and Participant II-6 in Family 8 showed widespread and abundant cytoplasmic aggregates of α -synuclein, primarily in oligodendroglia, in association with neurodegeneration in striatonigral and olivopontocerebellar structures. These findings confirmed the diagnosis of multiple-system atrophy.

PATIENTS WITH SPORADIC DISEASE AND CONTROLS

As with the multiplex families, the diagnosis of sporadic multiple-system atrophy was made on the basis of the current consensus criteria.⁸ A total of 363 patients with multiple-system atrophy and 520 controls were included in the Japanese series, 223 patients and 315 controls in the European series, and 172 patients and 294 controls in the North American series (persons of European or Hispanic descent living in North America) (Text S2 and Table S2 in the Supplementary Appendix). Ancestry was determined by self-report on a multiple-choice questionnaire. We also enrolled an independent series of 2383 Japanese controls.

ASSOCIATION WITH OTHER NEURODEGENERATIVE DISEASES

To determine the specificity of the association between variants in candidate genes and multiple-system atrophy, we enrolled 2728 Japanese patients with Alzheimer's disease, 659 with Parkinson's disease, and 634 with amyotrophic lateral sclerosis (ALS). Their demographic characteristics are provided in Text S2 in the Supplementary Appendix.

LINKAGE ANALYSIS AND WHOLE-GENOME SEQUENCING

We performed parametric and nonparametric linkage analyses using Affymetrix SNP 6.0 arrays and software for linkage analysis.^{16,17} The genomic DNA from Participant II-4 in Family 1 was subjected to four runs in an Illumina Genome Analyzer IIX (100-bp-long paired ends). We used BWA software¹⁸ and SAMtools sequence-alignment mapping¹⁹ with the default settings for alignment and variation detection against the human reference genome (National Center for Biotechnology Information build 36 [also known as hg18]).

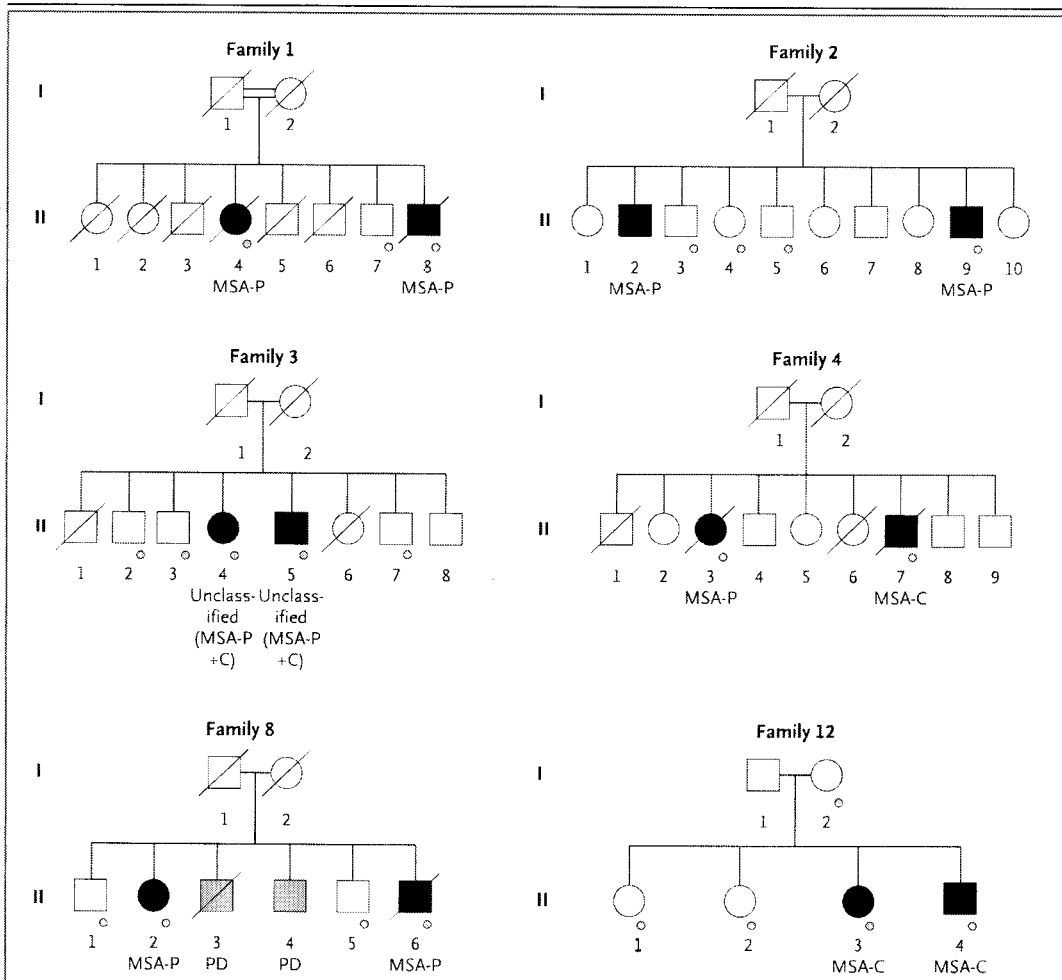


Figure 1. Pedigrees of Six Multiplex Families with Multiple-System Atrophy.

The affected siblings in Family 1 were born to consanguineous parents (first cousins).¹³ In this family, the two patients with multiple-system atrophy (Participants II-4 and II-8) also had retinitis pigmentosa, which was not present in the other siblings. The diagnosis of definite multiple-system atrophy in three patients (Participants II-4 and II-8 in Family 1 and II-6 in Family 8) was confirmed at autopsy. In Family 8, two siblings (Participants II-3 and II-4) of the affected family members had Parkinson's disease (PD). In Family 1, in which homozygous M128V-V393A mutations in *COQ2* were identified, the parents (Participants I-1 and I-2), who were obligate carriers of the mutation, showed no overt signs of parkinsonism, cerebellar ataxia, or autonomic dysfunction, according to family report. In Family 12, in whom compound heterozygous R387X/V393A mutations were identified, Participants I-1 and I-2 (obligate carriers of the mutations) and the heterozygous carrier (Participant II-2) showed no overt signs of parkinsonism, cerebellar ataxia, or autonomic dysfunction on examination by a neurologist. Squares represent male family members, circles female family members, black symbols family members with multiple-system atrophy, gray symbols family members with Parkinson's disease, open symbols unaffected family members, slashes deceased family members, and small circles family members for whom genomic DNA samples were available. MSA-C denotes multiple-system atrophy of the cerebellar type, MSA-P multiple-system atrophy with predominant parkinsonism, and unclassified MSA-P+C similarly predominant parkinsonian and cerebellar signs.

ANALYSIS OF *COQ2* AND OTHER GENES ASSOCIATED WITH COENZYME Q₁₀

On the basis of linkage analysis and whole-genome sequencing, we sequenced *COQ2* and the other 11 genes involved in the biosynthetic pathway for coenzyme Q₁₀ (*PDSS1*, *PDSS2*, *COQ3*, *COQ4*, *COQ5*,

COQ6, *COQ7*, *ADCK3*, *COQ9*, *COQ10A*, and *COQ10B*), using the Sanger method (Table S3 in the Supplementary Appendix).

We prepared samples of mutant human *COQ2* complementary DNA (cDNA) by means of site-directed mutagenesis (Table S4 in the Supple-

mentary Appendix). A yeast *coq2*-null mutant, the BY4741 Δ *coq2* strain, was transformed with pAUR123 (Takara Bio) containing the nonmutated or mutated human *COQ2* cDNA. We measured the growth rate in a medium with a nonfermentable carbon source by monitoring the optical density of a sample measured at a wavelength of 600 nm (OD_{600}). We used mitochondrial fractions prepared from lymphoblastoid cell lines with the QProteome Mitochondria Isolation Kit (Qiagen) as the enzyme source. *COQ2* activity (Enzyme Commission number, 2.5.1.39) was assayed as described previously.²⁰

COENZYME Q₁₀ LEVEL IN TISSUES

Using high-performance liquid chromatography, we measured levels of coenzyme Q₁₀ (ubiquinone-10 and ubiquinol-10) and free (unesterified) cholesterol in lymphoblastoid cell lines established from 152 patients with multiple-system atrophy and 76 controls and in cerebellum samples obtained on autopsy from 3 patients with multiple-system atrophy and 3 controls.²¹

STATISTICAL ANALYSIS

All results are presented as means and standard deviations. We used Student's t-test to evaluate the significance of differences in the mean age at disease onset between carriers and noncarriers of the *COQ2* mutation. We used Fisher's exact test to calculate the significance of the difference in allele frequencies between carriers and noncarriers, with contingency tables and standard methods used to compute odds ratios and corresponding 95% confidence intervals. We used the Kruskal-Wallis test, followed by the Steel test, to perform an analysis of variance. All statistical tests were two-sided, and a P value of less than 0.05 was considered to indicate statistical significance.

RESULTS

LINKAGE ANALYSIS OF FAMILIAL DISEASE

Parametric linkage analysis of the six family pedigrees revealed no single locus showing a linkage compatible with autosomal recessive inheritance. However, in the parametric linkage analysis allowing for heterogeneity, we detected several loci showing positive scores for heterogeneity logarithm of the odds (HLOD), indicating that more than one locus was involved in the different multiplex families (Fig. S1B in the Supplementary

Appendix). In particular, two regions on chromosome 4 showed the highest HLOD scores, exceeding 2.0. Results of nonparametric linkage analysis (Fig. S1C in the Supplementary Appendix) were consistent with those of parametric linkage analysis allowing for heterogeneity. Parametric linkage analysis of chromosome 4 in individual pedigrees revealed positive LOD scores in an overlapping region in four families (Family 1, Family 2, Family 4, and Family 12), with Family 1 having the highest LOD score of 1.93 (72.795 to 89.616 Mb) (Fig. S1A and S2A in the Supplementary Appendix). Thus, we selected Family 1 to undergo whole-genome sequencing.

SUSCEPTIBILITY GENE IN FAMILIAL DISEASE

Whole-genome sequencing of a sample obtained from Participant II-4, one of two affected members of Family 1, generated 187.5 Gb of short reads, with an average coverage of 58 \times and 3,492,429 single-nucleotide variants (SNVs) or insertions or deletions. We winnowed the 3,492,429 variants down to 4 by selecting SNVs that were located in the candidate regions defined on linkage analysis in Family 1 (regions with the highest LOD score spanning approximately 80 Mb in total), that were located in exons or splice sites, that were predicted to cause amino acid changes or changes in pre-messenger RNA splicing, and that were not registered in the database of single-nucleotide polymorphisms, build 130 (dbSNP130), indicating that the variants are extremely rare in the general population (Fig. S2B in the Supplementary Appendix). Each of these 4 SNVs is predicted to result in an amino acid substitution: K707R (c.2120A \rightarrow G) in *SHROOM3* (NCBI Reference Sequence, NM_020859.3), M128V (c.382A \rightarrow G) and V393A (c.1178T \rightarrow C) in *COQ2* (NCBI Reference Sequence, NM_015697.7), and R231G (c.691A \rightarrow G) in *SCEL* (NCBI Reference Sequence, NM_144777.2).

In the 180 Japanese control samples, we did not observe the SNV encoding the M128V variant but did observe SNVs encoding K707R in *SHROOM3*, V393A in *COQ2*, and R231G in *SCEL*, which were present on 3, 5, and 98 of 360 alleles, respectively. We therefore considered the SNP encoding M128V in *COQ2*, which encodes parahydroxybenzoate-polyprenyl transferase, an enzyme involved in the biosynthesis of coenzyme Q₁₀, as a candidate variant in conferring susceptibility to familial multiple-system atrophy.

Cosegregation analysis of samples from Family 1 revealed that the two affected family mem-

bers, Participants II-4 and II-8, carried the homozygous M128V-V393A variant in *COQ2*, and the unaffected sibling who was tested (Participant II-7) did not carry this variant (Fig. S2C in the Supplementary Appendix). Mutational analysis of *COQ2* in Family 12 revealed heterozygous mutations consisting of nonsense (R387X, c.1159C→T) and missense (V393A) variants in both affected siblings (Participants II-3 and II-4). Their mother (Participant I-2) was heterozygous for V393A, one unaffected sibling (Participant II-1) lacked this variant, and the other unaffected sibling (Participant II-2) was heterozygous for R387X. R387X was not observed in the 180 Japanese controls.

We did not detect variants of *COQ2* in the other four families (Families 2, 3, 4, and 8). Because *COQ2* encodes an enzyme essential for the biosynthesis of coenzyme Q₁₀, we further sequenced the other 11 genes in the biosynthetic pathway for coenzyme Q₁₀ (*PDSS1*, *PDSS2*, *COQ3*, *COQ4*, *COQ5*, *COQ6*, *COQ7*, *ADCK3*, *COQ9*, *COQ10A*, and *COQ10B*) in the remaining four families and in a previously described multiplex family¹⁴ but did not observe variants that cosegregated with disease.

COQ2 VARIANTS AND SPORADIC DISEASE

To investigate the involvement of *COQ2* variants in sporadic multiple-system atrophy, we extended the mutational analysis of *COQ2* to a Japanese series consisting of 363 patients with multiple-system atrophy and 520 controls. A common *COQ2* variant (rs6818847, predicted to result in an amino acid substitution, V66L (c.196G→T) with allele frequencies of 0.10 and 0.12 in the Japanese patients with multiple-system atrophy and controls, respectively, was not included in further analysis. Four patients with multiple-system atrophy carried two variants simultaneously (one carried an I147T [c.440T→C] and a nonmutated [NM] allele at codon 147 and V393A/NM at codon 393, one had R387Q [c.1160G→A]/NM at codon 387 and V393A/NM at codon 393, and two had V393A/V393A), whereas none of the controls had two variants of *COQ2* (Table 1). Sequencing of the subcloned mutated alleles confirmed that R387Q/V393A was present in a compound heterozygous state. We were unable to determine the phase of I147T/V393A, because the distance between I147T and V393A was too large to be amplified by means of polymerase-chain-reaction (PCR) assay in a single fragment, and samples

Table 1. *COQ2* Variants Found in Patients with Sporadic Multiple-System Atrophy in Japanese, European, and North American Series, as Compared with Controls.*

Genotype	Japanese Series		European Series		North American Series	
	Patients (N=363)	Controls (N=520)	Patients (N=223)	Controls (N=315)	Patients (N=172)	Controls (N=294)
P72L/NM	0	1	0	0	0	0
F79L/NM	0	0	1	0	0	0
P99H†/NM	0	0	0	0	1	0
S107T†/NM	0	0	1	0	0	0
R119H†/NM	0	0	0	0	0	1
I147T‡/V343A§	1	0	0	0	0	0
P157S†/NM	1	0	0	0	0	0
S163F†/NM	1	0	0	0	0	0
T317A‡/NM	0	0	1	0	0	0
S347C‡/NM	0	0	1	0	0	0
N386H/NM	0	1	0	0	0	0
R387Q†/V393A§	1	0	0	0	0	0
V393A§/NM	29	17	0	0	0	0
V393A§/V393A§	2	0	0	0	0	0

* NM denotes nonmutated.

† This variant was deemed to be severely deleterious on yeast complementation assay.

‡ This variant was deemed to be mildly deleterious on yeast complementation assay.

§ This variant had decreased *COQ2* activity on enzyme assay.

Table 2. Association between the COQ2 V393A Variant and Sporadic Multiple-System Atrophy in the Japanese Series.*

V343A Variant†	Patients with Multiple-System Atrophy			Patients with Other Neurologic Diseases		
	Patients (N=363)	Tier 1 Controls (N=520)	Tier 2 Controls (N=2383)	Alzheimer's Disease (N=2728)	Parkinson's Disease (N=659)	ALS (N=634)
Allele frequency — no./total no. (%)	35/726 (4.8)	17/1040 (1.6)	106/4766 (2.2)	109/5456 (2.0)	33/1318 (2.5)	31/1268 (2.4)
Heterozygous — no.	31	17	106	105	33	31
Homozygous — no.	2	0	0	2	0	0
		odds ratio (95% CI)	P value	odds ratio (95% CI)	P value	
		3.05 (1.65–5.85)	1.5×10 ⁻⁴	2.23 (1.46–3.32)	6.0×10 ⁻³	

* Odds ratios and P values are for the comparisons between patients with multiple-system atrophy and each of the two groups of controls (tier 1 and tier 2). ALS denotes amyotrophic lateral sclerosis, and CI confidence interval.
 † In the combined series of Japanese, European, and North American participants, functionally deleterious variants P99H, S107T, R119H, I147T, P157S, S163F, T317A, S347C, and R387Q (as determined on yeast complementation assay) were found in 8 of 1516 alleles (0.53%) in patients with multiple-system atrophy, as compared with 1 of 2258 alleles (0.05%) in controls (odds ratio, 11.97; 95% CI, 1.60 to 531.5; P=0.004).

of genomic DNA from the parents were unavailable. We found that 29 patients with multiple-system atrophy and 17 controls were heterozygous for the V393A variant. In addition, we detected four novel heterozygous variants: two in patients with multiple-system atrophy (P157S [c.469C→T] and S163F [c.488C→T]) and two in controls (P72L [c.215C→T] and N386H [c.1156A→C]).

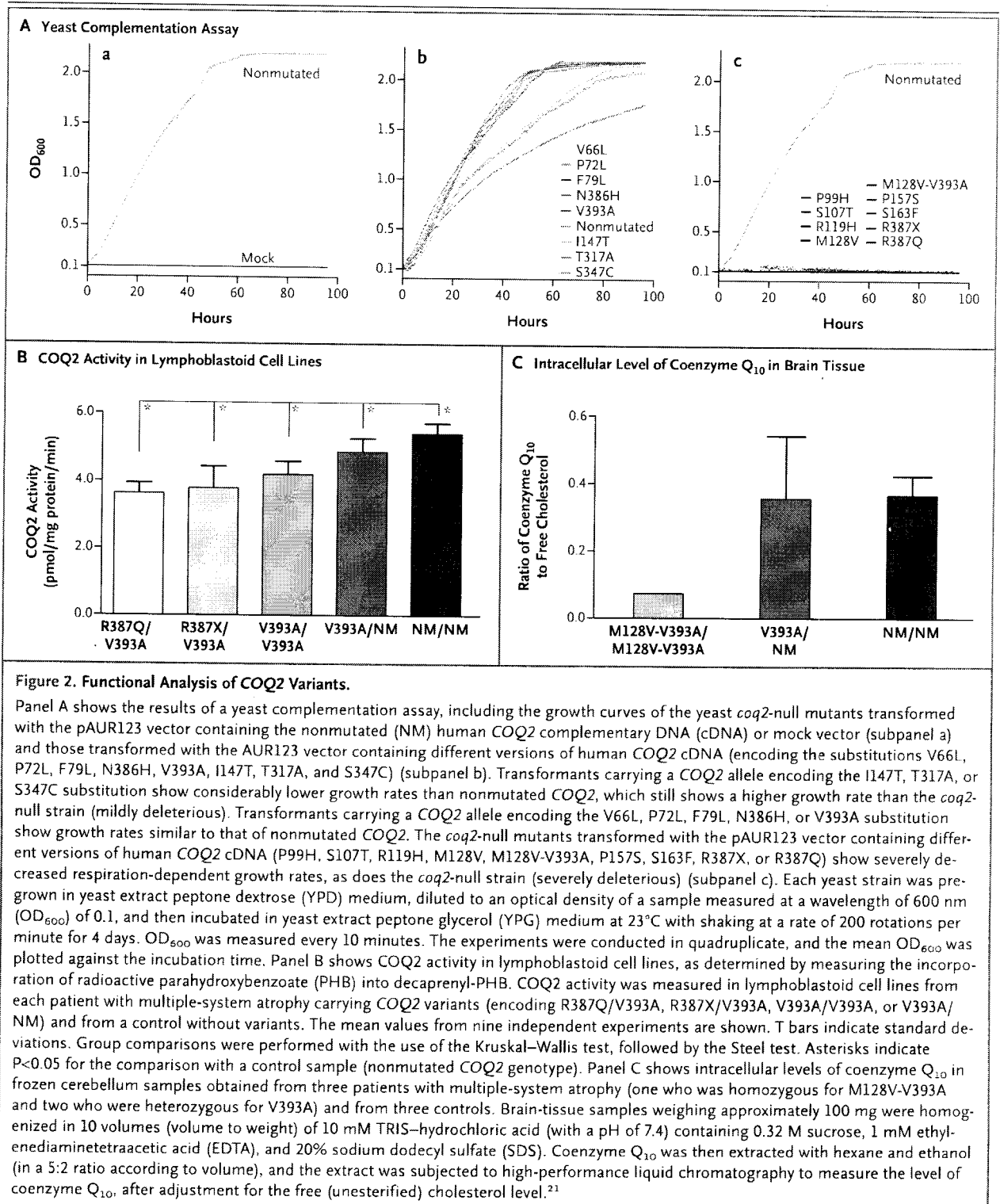
Of the COQ2 variants, the V393A variant is relatively common in the Japanese population. As shown in Table 2, we found that the V393A allele occurred in 35 of 726 alleles (4.8%) from Japanese patients with multiple-system atrophy and in 17 of 1040 alleles (1.6%) from Japanese controls (odds ratio for patients with multiple-system atrophy, 3.05; 95% confidence interval [CI], 1.65 to 5.85; P=1.5×10⁻⁴). Genotyping in the second series of 2383 Japanese controls showed that the V393A variant had an allele frequency of 2.2% (106 of 4766 alleles; odds ratio, 2.23; 95% CI, 1.46 to 3.32; P=6.0×10⁻⁵). Genotyping Japanese persons with other neurodegenerative diseases revealed that the V393A allele frequencies were 2.0% (109 of 5456 alleles) among patients with Alzheimer's disease, 2.5% (33 of 1318 alleles) among those with Parkinson's disease, and 2.4% (31 of 1268 alleles) among those with ALS. These allele frequencies did not differ significantly from those in the first or second set of controls, confirming the specificity of the V393A variant in patients with multiple-system atrophy. Two patients with Alzheimer's disease who were found to carry homozygous V393A mutations did not show any signs of parkinsonism, cerebellar ataxia, or autonomic dysfunction.

We then performed genotyping in the European and North American series of patients with multiple-system atrophy. In the European series, we found four singleton COQ2 variants (encoding amino acid substitutions F79L [c.235T→C], S1077T [c.320G→C], T317A [c.949A→G], and S347C [c.1039A→T]) among the patients, whereas none of the controls had any variants in COQ2. In the North American series, we found one variant (P99H [c.296C→A]) in a patient with multiple-system atrophy and one variant (R119H [c.356G→A]) in a control (Table 1). At the time of recruitment for the study, the carrier of R119H, who was 60 years old, had no signs of parkinsonism, cerebellar ataxia, or autonomic dysfunction, but this participant was unavailable for follow-up assessment. Intriguingly, the V393A

variant, a relatively common variant in the Japanese population, was not observed in patients with multiple-system atrophy or controls in either the European or the North American series.

FUNCTIONAL ANALYSIS OF MUTANT COQ2

To determine the functional effect of each variant on the mitochondrial aerobic energy production in which coenzyme Q₁₀ plays an essential



role in the electron transfer, we carried out functional complementation analysis by transforming the yeast *coq2*-null strain with nonmutated or mutated human *COQ2* cDNA (Fig. 2A). Transformants of the BY4741 Δ *coq2* yeast strain with the mutated *COQ2*, including transformants separately carrying the P99H, S107T, R119H, M128V, M128V-V393A, P157S, S163F, R387Q, and R387X alleles, showed severely decreased growth rates, similar to those observed in the *coq2*-null strain. In addition, transformants with mutated *COQ2*, including those with the variants encoding the I147T, T317A (c.949A→G), and S347C (c.1039A→T) substitutions, showed substantially lower growth rates than those expressing nonmutated *COQ2*, which had a higher growth rate than the *coq2*-null strain (mildly deleterious). The transformants with mutated *COQ2*, including transformants separately carrying the V66L, P72L, F79L, N386H, and V393A alleles, showed growth rates similar to those of the transformants expressing nonmutated *COQ2*. As described above, the yeast strain with M128V-V393A identified in Family 1 showed a severely decreased growth rate, whereas the strain with V393A had a growth rate similar to that of nonmutated *COQ2*, indicating that of the two variants, M128V primarily contributed to the impairment in *COQ2* function.

Focusing on the rare variants that were identified in the case-control series (Table 1), we found that nine variants (P99H, S107T, R119H, I147T, P157S, S163F, T317A, S347C, and R387Q) were mildly or severely deleterious. On combining all three series, eight variants (P99H, S107T, I147T, P157S, S163F, T317A, S347C, and R387Q) were identified in 758 patients with multiple-system atrophy, whereas only one variant (R119H) was found in 1129 controls (odds ratio, 11.97; 95% CI, 1.60 to 531.52; $P=0.004$) (Table 2 footnote). Yeast complementation analysis showed that the F79L variant, identified in a European patient with multiple-system atrophy, did not impair the growth rate. Lymphoblastoid cell lines from this patient were unavailable for further measurement of the activity of mutant *COQ2*, thus making it difficult to interpret the pathogenicity of this variant.

COQ2 ACTIVITIES IN LYMPHOBLASTOID CELL LINES

We measured *COQ2* activities in lymphoblastoid cell lines from patients carrying *COQ2* mutations, when available. We focused on the V393A variant because it is commonly associated with multiple-

system atrophy and showed an apparently normal growth rate in the yeast complementation assay. We determined *COQ2* activities in lymphoblastoid cell lines with *COQ2* variants R387Q/V393A, R387X/V393A, V393A/V393A, or V393A/NM and in a control without variants. The *COQ2* activities in the lymphoblastoid cell lines (V393A/NM) obtained from patients with multiple-system atrophy were significantly lower than those in the control cell lines. The *COQ2* activities in the cell lines from patients with multiple-system atrophy carrying two mutated *COQ2* alleles were further decreased (Fig. 2B).

CORRELATIONS BETWEEN GENOTYPE AND PHENOTYPE

The clinical features of patients with sporadic multiple-system atrophy carrying deleterious *COQ2* variants (as determined on yeast complementation assay and *COQ2*-activity measurement) and those of noncarriers are summarized in Table S5 in the Supplementary Appendix. The mean age at the onset of multiple-system atrophy among carriers was older than that among noncarriers ($P=0.002$). Among carriers, 34 had subtype C and 5 had subtype P. Among noncarriers, 468 had subtype C and 209 had subtype P. The subtype was unclassified in 42 noncarriers. The ratio of the number of patients with subtype C to the number with subtype P was significantly higher among carriers of *COQ2* variants than among noncarriers ($P=0.02$).

INTRACELLULAR COENZYME Q₁₀ IN LYMPHOBLASTOID CELL LINES

We measured intracellular coenzyme Q₁₀ levels in lymphoblastoid cell lines from patients with multiple-system atrophy and controls. The participants were grouped as follows: 3 patients with multiple-system atrophy carrying two variants (R387Q/V393A, R387X/V393A, and V393A/V393A), 16 patients carrying heterozygous V393A, 133 patients without variants, and 76 controls without *COQ2* variants (Table 3). Intracellular levels of coenzyme Q₁₀ in lymphoblastoid cell lines from patients with multiple-system atrophy who carried two variant alleles were substantially lower than levels in cell lines from controls without variants. Intracellular coenzyme Q₁₀ levels in patients who were heterozygous for V393A and in those without *COQ2* variants were not significantly lower than levels in controls without *COQ2* variants.

Table 3. Intracellular Levels of Coenzyme Q₁₀ in Lymphoblastoid Cell Lines, According to COQ2 Variant.*

Variable	Patients with Multiple-System Atrophy				Controls	
	R387Q/V393A	R387X/V393A	V393A/V393A	V393A/NM	NM/NM	NM/NM
No. of participants with variant	1	1	1	16	133	76
Ratio of coenzyme Q ₁₀ to free (unesterified) cholesterol†	2.19	2.58	1.86	3.38±0.53	3.41±0.74	3.48±0.75
Coenzyme Q ₁₀ level as a percentage of mean value in controls — %‡	62.9	74.1	53.4	97.1	98.0	100.0

* Plus-minus values are means ±SD. NM denotes nonmutated.

† The ratio of coenzyme Q₁₀ to free (unesterified) cholesterol reflects the intracellular level of coenzyme Q₁₀. Lower values indicate decreased levels of intracellular coenzyme Q₁₀, presumably reflecting decreased biosynthesis of coenzyme Q₁₀. To calculate the ratio, coenzyme Q₁₀ was measured in nanomoles per liter and free cholesterol in micromoles per liter.

‡ Lower values indicate decreased levels of intracellular coenzyme Q₁₀, as compared with the mean value in controls, presumably reflecting decreased biosynthesis of coenzyme Q₁₀.

COENZYME Q₁₀ IN BRAIN TISSUE

Only a limited number of brain-tissue samples from patients with multiple-system atrophy carrying COQ2 variants were available. Nevertheless, we measured coenzyme Q₁₀ in frozen brain tissues from three patients with COQ2 variants (one patient who was homozygous for M128V-V393A and two patients with V393A/NM) and from three controls without COQ2 variants (Fig. 2C). The levels of coenzyme Q₁₀ in patients who were homozygous for M128V-V393A were substantially lower than the levels in controls.

DISCUSSION

We identified homozygous or compound heterozygous COQ2 mutations in two of the six multiplex families with multiple-system atrophy, a finding that suggests a role of these mutations in the pathogenesis of familial disease. We further found that functionally impaired variants in COQ2 were associated with an increased risk of sporadic disease. In familial cases of multiple-system atrophy, linkage analysis strongly indicated locus heterogeneity in these families, and the identification of the causal variants in the remaining four families will require analyses such as whole-genome sequencing.

We found that a common variant (V393A) and multiple rare variants in COQ2 were associated with sporadic multiple-system atrophy. The V393A variant was found exclusively in the Japanese participants, with an allele frequency of 1.6 to 2.2%. The allele frequency of V393A in patients with multiple-system atrophy (4.8%) was signifi-

cantly higher than that in controls (1.6 to 2.2%) with odds ratios of 2.23 to 3.05. The modest risk of multiple-system atrophy that was associated with the common variant V393A suggests that V393A is a susceptibility factor rather than a causal factor for this disease. The odds ratio for the presence of deleterious rare variants was 11.97, which is much larger than that for V393A. Nonetheless, we should consider that these heterozygous variants in COQ2 are not necessarily causal but rather confer a strong susceptibility to sporadic multiple-system atrophy. Members of Family 1 and Family 12 who carried deleterious variants in the heterozygous state did not have clinical signs of multiple-system atrophy.

The ratio of patients with subtype C multiple-system atrophy to those with subtype P was higher among carriers of deleterious COQ2 variants than among noncarriers, which suggests that the cerebellum is more vulnerable to compromised COQ2 function than other regions of the central nervous system. Of the COQ2 variants that we detected, the V393A variant was the most prevalent and was exclusively found in Japanese participants. These findings may in part explain the clinical observations that subtype C is more prevalent than subtype P in the Japanese population⁹ but not in the European population¹¹ or the North American population.¹² However, there were only 35 carriers of deleterious COQ2 variants among 363 patients with multiple-system atrophy in the Japanese case series. In addition, the clinical presentations of the two patients with familial disease who had the highest mutational load were different: subtype P in the patients in Fam-

ily 1 and subtype C in the patients in Family 12. Thus, the genotypes of *COQ2* do not fully explain the clinical phenotypes.

Previous studies have shown evidence of mitochondrial respiratory-chain dysfunction or oxidative injury in patients with multiple-system atrophy.²²⁻²⁴ The combination of oxidative stress and overexpression of oligodendroglial α -synuclein has been reported to replicate the characteristics of this disease.²⁵⁻²⁸ Our findings suggest that impaired *COQ2* activity, which would be predicted to impair the mitochondrial respiratory chain and increase vulnerability to oxidative stress, causes susceptibility to multiple-system atrophy. A primary deficiency of coenzyme Q₁₀ that is caused by *COQ2* mutations has been described as an infantile-onset multisystem disorder and a nephropathy in several families.^{29,30} The clinical presentation of these affected family members, however, differed markedly from the presentations of patients with multiple-system atrophy, perhaps because the decrease in *COQ2* activity associated with the mutations in patients with multiple-system atrophy appears to be milder than that observed in patients with a primary deficiency of coenzyme Q₁₀.

Previous approaches to identifying susceptibility genes have used genomewide association studies or candidate-gene approaches.³¹⁻³³ Our identification of rare *COQ2* variants was accomplished by starting with multiplex families and then extending the analysis to patients with sporadic multiple-system atrophy, reflecting an alternative approach to the elucidation of genetic variants with strong effect sizes in an apparently nongenetic disorder.³⁴

From the therapeutic viewpoint, oral supplementation with coenzyme Q₁₀ may be helpful in treating multiple-system atrophy, particularly for patients with susceptibility-conferring *COQ2* variants. The safety and side-effect profile of high-dose supplementation with coenzyme Q₁₀ have been well established.^{35,36}

ADDENDUM

Human *COQ2* contains four ATG codons in exon 1. Among the four potential translation initiation codons in exon 1, the protein isoform starting at the fourth ATG codon has been adopted as the canonical sequence of the *COQ2* protein in the UniProt database (Q96H96, <http://www.uniprot.org/uniprot/Q96H96>). In this study, we focused entirely on *COQ2* variants after the fourth ATG codon.

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APPENDIX

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SNCA Variants Are Associated with Increased Risk for Multiple System Atrophy

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Abstract

To test whether the synucleinopathies Parkinson's disease and multiple system atrophy (MSA) share a common genetic etiology, we performed a candidate single nucleotide polymorphism (SNP) association study of the 384 most associated SNPs in a genome-wide association study of Parkinson's disease in 413 MSA cases and 3,974 control subjects. The 10 most significant SNPs

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were then replicated in additional 108 MSA cases and 537 controls. SNPs at the SNCA locus were significantly associated with risk for increased risk for the development of MSA (combined $p = 5.5 \times 10^{12}$; odds ratio 6.2).

Introduction

Multiple system atrophy (MSA) and Parkinson's disease (PD) are progressive neurodegenerative disorders characterized neuropathologically by deposition of abnormally phosphorylated α -synuclein. In PD, the aggregates are typically found in neurons as Lewy bodies, whereas in MSA, α -synuclein is deposited predominantly in the form of glial cytoplasmic inclusions.¹ These observations suggest that PD and MSA share a common pathogenic mechanism.

Although MSA appears to occur sporadically in the community, a number of recent observations have implicated genetic factors in the pathogenesis of the disease. First, neurological signs of parkinsonism are more common in relatives of MSA patients.^{2,3} Second, affected members within families with SNCA duplication or triplication manifest clinical and pathological features similar to MSA.⁴⁻⁶ Lastly, there are reports of MSA occurring within families, typically with an autosomal recessive inheritance pattern.^{7,8}

We recently completed a genome-wide association study of 1,713 white PD cases and 3,974 white control subjects. Based on this initial cohort, 384 single nucleotide polymorphisms (SNPs) that were most associated with increased risk for development of PD were selected for further testing in an additional cohort of PD cases and control subjects, and we have presented these findings separately.⁹ To test the hypothesis that MSA and PD share a common genetic causative factor, we tested the same 384 SNPs identified by our PD genome-wide association study in 413 MSA cases and 3,974 healthy control subjects. To confirm our findings, we then replicated the 10 most significant SNPs from this initial screening of MSA cases in an additional cohort of 108 MSA cases and 537 healthy control subjects. Our analysis demonstrated that genetic variants at the SNCA locus coding for α -synuclein were highly significantly associated with increased risk for development of MSA.

Subjects and Methods

Samples

The initial screening cohort consisted of 413 white MSA cases and 3,974 white healthy control subjects. The cases were a mixture of pathologically certain MSA patients ($n = 99$) and clinically probable or possible cases ($n = 314$). A total of 283 of 413 MSA cases were included from collaborating centers of the European MSA study group (www.emsa-sg.org). The replication stage was composed of an independent cohort of 108 clinically probable white MSA cases and 537 white healthy control subjects. Diagnosis of patients was based on consensus criteria that Gilman and colleagues¹⁰ established. Clinical features and collection sites of cases and control subjects are described in Supplemental Tables 1 and 2. The study was approved by each respective institutional review board, and written informed consent was obtained for each participant.

Genotyping

Genotyping of the 384 SNPs selected for the initial screening stage was performed using custom-made GoldenGate assays on a Veracode platform as per the manufacturer's instructions (Illumina, San Diego, CA). Raw genotype data were analyzed using Beadstudio software (version 3.1.0; Illumina).

For the replication stage, genotyping was performed by polymerase chain reaction followed by direct sequencing on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) (primer sequences listed in Supplemental Table 3). Genotype information for the control cohort used in the replication stage was extracted from publicly available data of 537 British healthy control subjects who had been previously genotyped on Illumina 610Y SNP chips.

Statistical Analysis

Statistical analysis was performed using PLINK software (v1.04).¹¹ For the screening stage, samples with a call rate less than 90% were excluded from analysis ($n = 13$ cases and 83 control subjects). SNPs with a minor allele frequency less than 0.01 ($n = 3$), SNPs with significant departure from Hardy–Weinberg equilibrium ($p = 0.001$; $n = 29$), SNPs with a missingness rate greater than 5% ($n = 26$), or SNPs with inaccurate clustering ($n = 2$) were excluded from analysis (15 SNPs failed more than one quality-control criterion). Each of the remaining 339 SNPs was then tested for association under allelic, genotypic, dominant, recessive, and trend models, and the lowest p value was calculated for each SNP (p_{min}). Applying the Bonferroni method to correct for multiple testing, the threshold p value for significance was 2.6×10^{-5} (two-sided α of 0.05 divided by [384 SNPs multiplied by 5 models]).

For the replication stage, one SNP was excluded because of departure from Hardy–Weinberg equilibrium in control subjects ($p = 0.01$). The remaining SNPs were tested for association under a recessive model, because this model was the best fit in the screening stage. Based on Bonferroni correction for multiple testing, a p value less than 0.005 was considered significant (two-sided α of 0.05 divided by 10 SNPs tested). The power of this cohort to replicate loci at this significance level with the odds ratios observed in the screening stage is shown in Supplemental Figure 2.

Results

Screening Stage

A total of 384 SNPs were genotyped in a cohort of 413 MSA cases and 3,974 control subjects. After quality-control filters were applied, 339 SNPs were tested for association with disease in a final dataset of 400 cases and 3,891 control subjects under allelic, genotypic, dominant, recessive, and trend models (results of the screening stage are shown in the Table and in Supplemental Figure 1).

Replication Stage

To replicate these findings, we genotyped the 10 most significantly associated SNPs identified in the screening stage in an independent, additional cohort of 108 MSA samples and 537 control samples (see the Table 1). Sequence analysis demonstrated a likely genotyping error for rs10515822; reexamination of cluster plots confirmed this error, and this SNP was removed from further analysis. Applying a recessive model, we observed highly significant associations exceeding the Bonferroni threshold for two of these SNPs, namely, rs11931074 ($p = 1.6 \times 10^{-4}$) and rs3857059 ($p = 1.3 \times 10^{-6}$). When data from the replication stage were combined with data from the screening stage, the p value for rs11931074 was 5.5×10^{-12} (odds ratio for homozygous risk allele carriers = 6.2 [95% confidence interval [CI]: 3.4–11.2]), and for rs3857059 was 2.1×10^{-10} (odds ratio for homozygous risk allele carriers = 5.9 [95% CI: 3.2–10.9]) (see Supplemental Table 4 for details). These two SNPs are in complete linkage disequilibrium ($r^2 = 1.0$ in the Centre d'Etude du Polymorphisme Humain HapMap population from Utah), and lie in intron 4 of SNCA (rs3857059) and downstream of SNCA (rs11931074) (Fig1). None of the remaining eight SNPs reached significance in the replication stage or in the combined analysis.

Analysis of Pathology-Proved Multiple System Atrophy Cases

To exclude the possibility that PD cases mistakenly clinically diagnosed as MSA might be falsely driving the association with SNCA, we analyzed the SNPs rs11931074 and rs3857059 in pathology-proven MSA cases and healthy control subjects (n = 92 cases and 3,891 control subjects after quality-control filtering). Both SNPs remained significantly associated with increased risk for development of MSA (recessive model p value for rs11931074 = 1.4×10^{-11} ; p value for rs3857059 = 4.9×10^{-6} ; see Supplemental Table 5).

Analysis of Clinical Multiple System Atrophy Subtypes

From available records, 136 patients could be unequivocally assigned to the MSA-P subtype, and 75 patients were MSA-C cases (see Supplemental Table 2 for further details on these cohorts). An analysis in these subgroups could not detect the association between SNCA variants and increased risk for development of MSA (MSA-P: rs11931074, p = 0.194; rs3857059, p = 0.183; MSA-C: rs11931074, p = 0.075; rs3857059, p = 0.069; recessive model using Fisher's exact test), probably because of lack of power in the relatively small subgroups. However, this result also suggests that the association is not driven just by one MSA sub-phenotype.

Discussion

In this study, we have demonstrated that genetic variants within the SNCA locus are associated with increased risk for development of MSA. These data represent the first genetic variants convincingly identified for patients with MSA. This study is important in that genetic factors play a greater role in the pathogenesis of MSA, which entity primarily suggests thought of as sporadic in occurrence. The veracity of our findings is underscored by the strength of the association that clearly exceeded the conservative Bonferroni threshold for statistical significance, by the successful replication of our findings in an independent cohort, and by the role that SNCA is already known to play in the disease process based on neuropathological findings.⁴⁻⁶

Previous studies (including sequencing of SNCA coding sequence, gene dosage measurements, microsatellite testing, and haplotype studies) have failed to identify significant association of SNCA variants with MSA.¹²⁻¹⁶ These negative results can be explained by the smaller sample sizes of these studies, and by the fact that none of the SNCA risk variants identified in our study was tested. Our replication of the association between SNCA variants and MSA in an independent patient and control cohort indicates that population stratification was unlikely to be falsely driving the finding. The failure to replicate our findings in MSA-P and MSA-C clinical subgroups was likely due to small sample size and the diagnostic uncertainty inherent to clinical criteria.¹⁷ A combination of these factors would negatively impact the power to detect association within these patient subsets, and studies of larger cohorts will be required to dissect the true pathogenic role of SNCA variants within each of these clinical categories. In contrast, analysis in the smaller, but diagnostically accurate, subset of pathology-proven MSA cases clearly demonstrates that SNCA variants are associated with increased risk for disease.

The significant associations with increased risk for MSA were most clearly observed under the recessive model. However, it is possible that the relatively small size of our case-control cohort was powered only to identify individuals carrying two risk alleles, but that an undetected additive risk at these loci exists. Additional studies involving larger patient cohorts are required to determine whether persons with a single copy of the risk allele are at increased risk for development of MSA.

How does genetic variation at the SNCA locus confer an increased risk for development of MSA? Previous sequence analysis of SNCA coding sequence failed to identify pathogenic mutations; thus, direct alteration of the amino acid sequence is considered an unlikely mechanism of disease.^{12,16} The most plausible explanation, therefore, would be a change in gene expression regulation. This explanation is supported by the observation that duplication or triplication of SNCA leads to glial cytoplasmic inclusion formation in the brains of affected individuals, and that in some subjects, the clinical presentation resembles a MSA phenotype.⁴⁻⁶ A modest alteration in gene expression levels, although pathogenic in a given individual, may have escaped detection in previous SNCA expression studies of small sample size.¹⁸⁻²⁰ The identified risk variants may also alter the splicing pattern of SNCA in a pathogenic manner, or alter SNCA messenger RNA processing, or additional genetic factors may be responsible for the different manners of synuclein accumulation in PD and MSA.

How do the results of our candidate SNP association study in MSA compare with our genome-wide association study in PD? We identified significant association with the SNCA locus in both diseases.⁹ The odds ratio associated with carrying a single risk allele of the SNCA SNP rs3857059 was 1.3 in both diseases (95% CI in PD: 1.2-1.5; 95% CI in MSA: 1.1-1.6), whereas the odds ratio for homozygous carriers was 3.8 (95% CI: 2.4-5.9) in PD and 5.9 (95% CI: 3.2-10.9) in MSA.

In summary, our study has conclusively demonstrated that genetic variants in SNCA play a role in the pathogenesis of MSA, and that these genetic factors overlap with those found in PD. These data support the general notion that variability at the gene that encodes the major pathologically deposited species is a risk factor in neurological diseases involving protein deposition²¹ but highlights that often large sample sizes are required to see such an effect. Additional genetic loci undoubtedly remain to be identified in the pathogenesis of this fatal neurodegenerative disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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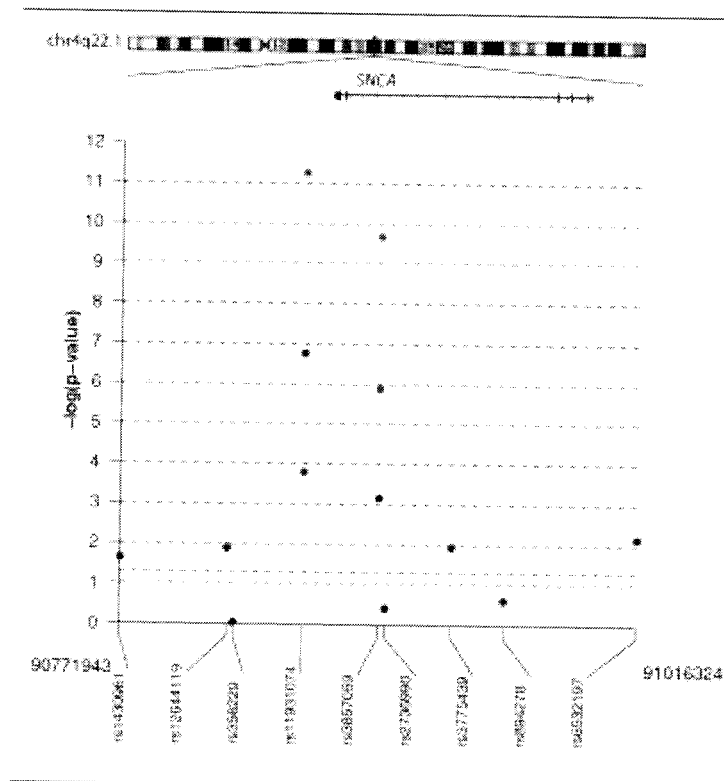


Fig. Location of the association signal at the SNCA locus on chromosome 4q22.1

Association signals are shown for all single nucleotide polymorphisms (SNPs) genotyped in (A) screening-stage samples (black circles), (B) replication-stage samples (blue circles), and (C) for combined screening- and replication-stage samples (red circles). The most associated SNPs, rs11931074 and rs3857059, lie in or near the SNCA gene, and are in complete linkage disequilibrium. The plot were generated using the SNP. plotter package within R version 2.6.1.

Table
Nine Most Significantly Associated Single Nucleotide Polymorphisms

SNP ID	Chromosome	Gene	Risk allele	Screening stage		Replication stage		Combined	
				P min (test model)	OR (95% CI) [RR vs (RP+PP)]	P recessive	OR (95% CI) [RR vs (RP+PP)]	P recessive	OR (95% CI) [RR vs (RP+PP)]
rs11931074	4q22.1	Downstream of SNCA	T	1.7E-07(recessive) ^b	5.4 (2.7-11.1)	1.6E-04 ^a	6.6 (2.15-19.93)	5.5E-12	6.2 (3.4-11.2)
rs3857059	4q22.1	SNCA	G	6.9E-04(recessive)	3.8 (1.7-8.5)	1.3E-06 ^a	9.8 (3.20-29.78)	2.1E-10	5.9 (3.2-10.9)
rs9480154	6q25.1	Downstream of PPP1R14C	A	1.6E-05(recessive) ^b	5.0 (2.2-11.2)	0.99	1.0 (0.12-8.81)	1.3E-04	3.9 (1.8-8.2)
rs2794256	6q22.31	LOC728727	T	1.7E-03(recessive)	1.7 (1.2-2.5)	0.17	1.6 (0.81-3.19)	4.0E-04	1.7 (1.3-2.4)
rs2042079	2p24.2	Intergenic	A	2.7E-03(recessive)	1.7 (1.2-2.5)	0.21	1.6 (0.77-3.18)	8.0E-04	1.7 (1.3-2.4)
rs13139027	4p16.2	Upstream of MSX1	A	2.5E-03(recessive)	3.9 (1.5-10.1)	0.53	1.5 (0.41-5.63)	1.8E-03	3.2 (1.5-6.9)
rs2515501	8p23.2	MCPHI	T	6.5E-04(recessive)	2.4 (1.4-4.1)	0.45	0.6 (0.13-2.52)	7.0E-03	1.9 (1.2-3.2)
rs2896159	7q31.2	Intergenic	T	3.0E-03(recessive)	0.7 (0.5-1.1)	0.38	1.3 (0.73-2.26)	0.43	1.3 (1.1-1.6)
rs2856336	12p13.2	ETV6	C	1.6E-08(recessive) ^b	4.6 (2.6-8.3)	0.12	— ^c	2.4E-05	3.1 (1.8-5.5)

^a Exceeded Bonferroni significance threshold in the replication stage (i.e., 0.05/10 = 0.005).

^b Exceeded Bonferroni significance threshold for multiple testing in the screening stage (i.e., 0.05/[384*5] = 2.6E-05).

^c Unable to calculate odds ratio (OR) because of low allele frequency in cases. SNP = single nucleotide polymorphism, CI = confidence interval, R = risk allele, P = protective allele, HWE = Hardy-Weinberg equilibrium.

SHORT REPORT

Probable multiple system atrophy in a German family

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Multiple system atrophy (MSA) is a neurodegenerative disorder of unknown aetiology. A possible underlying genetic component has not yet been identified. A family is reported with phenotypic MSA and probable autosomal dominant inheritance. The patients presented initially with either parkinsonian or cerebellar signs, and developed severe autonomic failure and typical atrophy of the brain stem and cerebellum in the course of the disease.

Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder of unknown aetiology, clinically characterised by poorly levodopa responsive parkinsonism with or without cerebellar dysfunction, in combination with autonomic failure.¹ α -Synuclein positive oligodendroglial inclusions are the specific neuropathological hallmark of MSA. Like idiopathic Parkinson's disease, MSA might therefore be classified as a sporadic synucleinopathy.² While both autosomal dominant and recessive traits have been identified in Parkinson's disease, no familial cases of MSA have yet been reported. We describe a family with a phenotype typical of MSA in two successive generations.

METHODS

All family members were examined by two of us (UW and TSH), using the unified multiple system atrophy rating scale (UMSARS) and the unified Parkinson's disease rating scale (UPDRS). All had standardised autonomic testing, and magnetic resonance imaging (MRI) was undertaken in nine family members, the protocol including T1-TSE three dimensional volume datasets and T2 weighted images, as described previously.¹ The two affected patients underwent additional single photon emission computed tomography (SPECT) with ¹²³I-FP-CIT and ¹²³I-IBZM.

RESULTS

The family comes from northern Germany. There are 14 living members in three generations, without any history of consanguinity (fig 1).

I₁ first presented at age 68 years with akinetic parkinsonism which responded well to levodopa treatment for approximately eight years. Urinary incontinence and orthostatic dysfunction developed within two years, and ataxia of gait and stance within six years after the initial diagnosis. On examination (aged 77), she showed no cognitive impairment. Ataxia of gait and stance was severe and she was unable to walk unaided. She had a smooth expressionless face, severe dysarthria, upper and lower limb ataxia, symmetrical brisk tendon reflexes, and cogwheel rigidity, but no plantar responses (UMSARS scores: I (activities of daily living), 35; II (motor examination), 43; UPDRS III, 39). Blood pressure was 140/100 mm Hg recumbent and 100/60 mm Hg upright, the change of posture producing giddiness. MRI showed severe brain stem and cerebellar atrophy with a hot cross bun

sign (fig 2). Only moderate changes were present in the putamen.

II₁ first presented at age 46 years with ataxia of gait. A partial (urge) incontinence, orthostatic dysfunction, and mild right sided parkinsonism developed within two years after the occurrence of ataxia. On examination (age 49), she showed no cognitive impairment. She had mild ataxia of gait and stance and mild upper and lower limb ataxia, moderate dysarthria, symmetrical brisk tendon reflexes, and mild cogwheel rigidity of the right arm, but no plantar responses (UMSARS scores: I (activities of daily living), 11; II (motor examination), 18; UPDRS III, 19). Blood pressure was 130/90 mm Hg recumbent and 100/90 mm Hg upright. MRI showed predominant cerebellar and moderate brain stem atrophy with a hot cross bun sign (fig 2).

Subjects II₂₋₄ (aged 48-55) all had completely normal neurological examinations except for a minimal ataxia of gait in II₂ when walking in tandem with eyes closed, and a corresponding discrete atrophy of the upper vermis on MRI (not shown).

Subjects III₁₋₃ (aged 21-28), the three children of II₁, had completely normal neurological examinations and MRIs (not shown).

I₁ and II₁ underwent SPECT with ¹²³I-FP-CIT and ¹²³I-IBZM. Both showed an asymmetrical massive reduction of the presynaptic dopamine transporter and a moderate loss of dopamine D2 receptors (data not shown). Extensive laboratory testing was undertaken in I₁ and II₁: all routine variables, including caeruloplasmin and urine copper, were within the normal range; CSF samples were normal, without oligoclonal bands. Genetic testing excluded spinocerebellar ataxia (SCA) types 1-3, 6, 7, and 17 in I₁ and II₁.

DISCUSSION

This is the first description of a family with the clinical phenotype of probable MSA, corresponding morphological changes on MRI, and a probable autosomal dominant inheritance. In 1964, before the recognition of MSA as a disease entity, Lewis described a family with orthostatic hypotension as the leading symptom.⁴ However, while two family members had ataxia and parkinsonism and might have fulfilled the current criteria of probable or possible MSA, a common feature in that family seemed to be prominent amyotrophy and rather slow disease progression, with a disease duration of more than 30 years in one case.¹ In addition, the predominant symptom in two cases was diarrhoea, which could point to coeliac disease. Diarrhoea, autonomic failure, and cerebellar degeneration are also observed in hereditary neuronal nuclear inclusion disease (NIID), even though at least in some cases NIID symptoms may start in childhood.⁵

Although a multigenic aetiology of MSA is often suspected, the evidence for a genetic component is rather weak, and families with MSA have not been described up to now. A family with necropsy proven Parkinson's disease and necropsy proven MSA was reported by Shimo *et al.*⁶ but the significance of that combination remains unclear. The

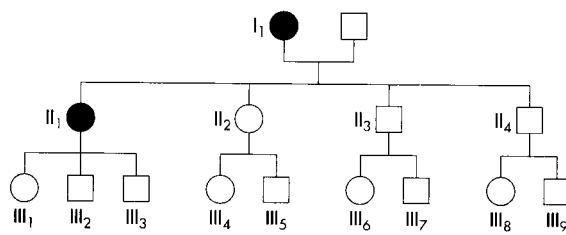


Figure 1 Patient pedigree.

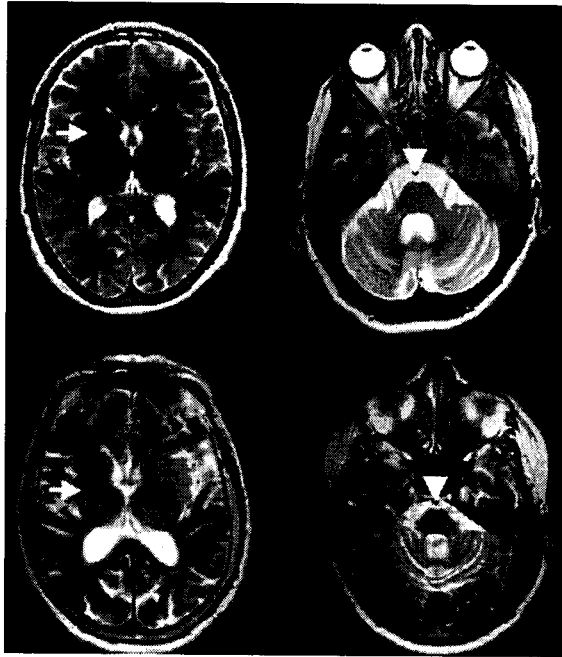


Figure 2 T2 weighted horizontal magnetic resonance images at the level of the basal ganglia (left) and the brain stem (right): bottom, I1; top, I11. Note the atrophy of the cerebellar peduncle with gliosis and the hot cross bun sign in the brain stem (arrowheads). Only moderate gliosis is present at the outer rim of the putamen (arrows).

probability that the cases encountered occurred by chance alone is rather low. The standardised prevalence rates of MSA are estimated to be 4 or 5 in 100 000.⁷ Thus the probability of finding two patients with probable MSA in a family of 14 members by chance alone is as low as 0.0006. A genetic predisposition for MSA was suggested by an epidemiological study by Nee *et al.*, who reported a higher frequency of symptoms and neurological diseases in first degree relatives of 60 MSA cases than in controls (23% in MSA cases *v* 10% in controls).⁸ Although a small study reported an association between MSA and a mutant allele of the CYP11D6 gene, a subsequent analysis did not find an association between MSA and genetic variants of the genes coding for CYP11D6, apoE, the receptor for IGF-1, ciliary neurotrophic factor (CNTF), the H5 pore region of the human homologue of the weaver mouse gene, or HLA-A32.^{9–11} Also, no mutations were

identified in the coding region of the α -synuclein gene.^{11–13} Neither CAG trinucleotide repeat expansions of spinocerebellar ataxia types 1–3, 6–8, and 12, nor frataxin mutations were found in 20 patients with possible or probable MSA.¹² In our family none of the known SCA mutations was found, although the marked difference in age at disease onset in the two affected family members (*anticipation*) could point to an as yet unidentified trinucleotide repeat disorder.

Identification of the present family suggests that MSA—although considered a sporadic disease—may be inherited in rare instances. As with Parkinson's disease, genetic analysis of larger families might provide clues to the suspected genetic basis of MSA.

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