脳血流SPECTおよびFDG-PET：
側頭葉後方部～頭頂連合野，後帯状回～楔前部の集積低下(後方優位低下型)
F-18 DOPA-PET：
両側線条体の集積は正常(ドーパミン合成能：シナプス節前機能は正常)
I-123 FP-CIT SPECT：
両側線条体の集積は正常(ドーパミントランスポーターは正常)
I-123 MIBG心交感神経シンチグラフィ：
心集積は正常(節後性交感神経機能は正常)
C-11 PIB-PETによるアミロイドイメージング：
陽性

問題4　認知症をきたす疾患の脳循環代謝所見として誤っているのはどれか。1つ選べ。(第8回　問6)

a. アルツハイマー型認知症においても脳血管障害のようなcrossed cerebellar diaschisisがみられることがある。
b. アルツハイマー型認知症の初期では海馬の血流低下が特徴的である。
c. 前頭側頭型認知症でも頭頂側頭連合野の血流が低下しうる。
d. 皮質基底核変性症では認知症の症状を呈するものはアルツハイマー型認知症の血流代謝低下パターンと同様の所見を示すが左右差が強いこと，基底核・視床・一次感覚運動野の血流代謝が低下するのが特徴的である。
e. レビー小体型認知症ではアルツハイマー型認知症の血流代謝低下パターンに加え，一次視覚野の血流代謝低下がみられることが特徴的である。

正解：b

AD初期では，後帯状回から楔前部および後方連合野の血流低下が特徴的である。ただし，80歳代以降の高齢発症のADでは，海馬領域の低下が目立つ例もあることは注意が必要である。

FTLDでは，頭頂葉下部(頭頂側頭連合野)に血流低下がおよぶことも少なくない。

問題5　レビー小体型認知症の画像所見で正しいのはどれか。

(1)脳血流SPECTによる線条体の血流低下
(2)^{123}I-iomazenilによる側頭葉内側の集積低下
(3)MRIによる後頭葉の萎縮
(4)^{123}I-β-CITによる線条体の集積低下
(5)^{123}I-MIBG心交感神経シンチグラフィによる心集積低下

正解：4，5

DLBでは，一般に線条体の血流，糖代謝は大脳皮質に比し高い。

問題6　^{11}C-PIB PETについて正しいのはどれか。1つ選べ。(第8回　問8)

a. 脳内で血液脳関門は通過しない。
b. レビー小体に特異的に強く集積する。
c. 集積陽性例では確実にAlzheimer型認知症と診断できる。
d. MCI(mild cognitive impairment)では陽性例はほとんどいない。
e. 健常者においてもアポリポプロテインE4(ApoE4)保有者では非保有者と比べて陽性率が高い。

正解：e

C-11 PiBはチオフラビン誘導体で，非常に脂溶性の高い化合物で，良好なBBB通過性を示す。そのため，白質への非特異的な集積を認める。撮像は通常，投与50分後から20分間(J-ADNIプロトコール)の撮像をおこなう。レビー小体やリン酸化タウ蛋白への親和性は低い。アルツハイマー病は，病理学的に，脳内アミロイド凝集体(Aβ)と神経原線維変化，神経細胞脱落により診断される。C-11 PiB陽性は，脳内アミロイドの蓄積を示すものであり，神経原線維変化の存在を示すものではない。PiB陰性は，Aβの蓄積はほとんどないことを意味し，「アルツハイマー病である可能性が極めて低い」ことを示唆する所見である。MCI例では，アルツハイマー病と同程度の集積を認める陽性例と皮質への集積を認めない陰性例に別れ，陽性率はおおよそ60－70%であり，陽性例は，アルツハイマー病への移行のリスクが高いとされる。健常者におけるPiB陽性率は，おおよそ20－30%程度で，ε4アレルとの相関を認め，アポリポプロテインE4(ApoE4)保有者では非保有者と比べて陽性率が高い。

リレー講座

診療に役立つ核医学の基本－専門医試験も見すえ－「脳神経核医学　臨床編その2」

百瀬　敏光　MOMOSE Toshimitsu

《はじめに》

前号の2013年1月号(Vol. 46, No. 1)において，「脳神経核医学　臨床編その1」として(1)脳血管障害，および(2)認知症と血流・代謝，アミロイドイメージングについて述べた。本編は「その2」として，(3)神経伝達機能イメージング，(4)てんかん，(5)脳腫瘍について解説する。

《神経伝達機能イメージング》

神経伝達機能イメージングとは，神経伝達物質の生合成，シナプス小胞での貯蔵，放出，受容体への結合，分解，再取り込みなど，シナプスでの化学的神経伝達の基盤となる様々な機能を，シナプスにおける特定の分子構造と結合する化合物(分子プローブ)を陽電子(ポジトロン)放出核種または単一光子(ガンマ線)放出核種により標識した放射性化合物を体内投与し，その動態をPETまたはSPECT装置を用いて計測することにより画像化する技術である。1983年，Henry Wagner Jr.らによってC-11 N-methyl spiperoneを用いて，ヒトで，はじめてPETによるドーパミン(D2 like)受容体が画像化された。その後，PET用分子プローブを中心に，様々な放射性化合物が開発され，シナプス前終末における神経伝達物質の合成能，シナプス間隙中に存在する分解酵素の活性，シナプス後膜に存在する受容体，細胞膜上の再取り込み部位，シナプス小胞膜上に存在するトランスポーター等の画像化に成功した。パーキンソン病を中心とした神経変性疾患における節前機能であるドーパミン合成能や節後機能であるドーパミンD2受容体結合能，うつ病，統合失調症など精神疾患における治療薬による受容体占有率など精神薬理学的側面からの臨床研究が進み，新たな知見が得られている。また，モノアミン系を中心に細胞膜型トランスポーターや小胞型トランスポーターに結合する放射性リガンドの開発が進み，特にドーパミンやセロトニンのシナプス前膜におけるトランスポーターは，主としてモノアミン系神経細胞の変性マーカーとして有用性が高く，汎用性の高いI-123標識SPECT用トレーサを用いて画像化され，臨床に広く利用されはじめている。現在，保険診療として行われているものは難治性てんかんにおける焦点診断のための中枢性ベンゾジアゼピン系受容体診断薬である^{123}I-iomazenilのみであるが，海外で臨床利用されている放射性医薬品および国内で研究用に利用されている放射性化合物についても重要なものについて解析法，至適撮像時間等を理解しておく必要がある。

問題7　脳のイメージング対象と使用する薬剤の組み合わせで誤っているのはどれか。
(第5回　総論　問題5)

a. アミロイド沈着 —— ^{11}C-PIB
b. 中枢性ベンゾジアゼピン受容体 —— ^{123}I-iomazenil
c. ドーパミンD1受容体 —— ^{11}C-raclopride
d. ドーパミントランスポーター —— ^{123}I-β-CIT
e. アポトーシス初期細胞 —— ^{99m}Tc-annexin V

正解：c

C-11 raclopride はドーパミンD2 like受容体に対し高い親和性をもつD2 antagonistである。D1 like受容体への高い親和性をもつ放射性薬剤は，C-11 SCH23390である。

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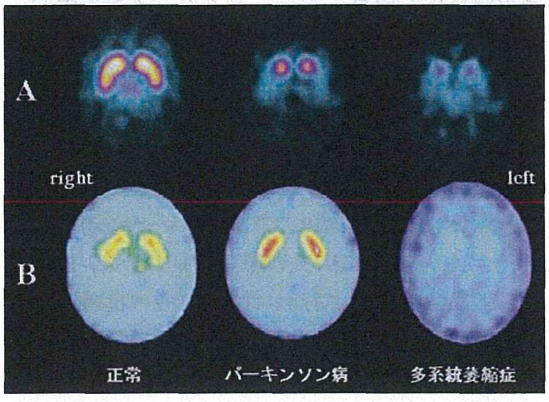
問題8　図は正常者およびパーキンソン病・多系統萎縮症患者における神経伝達機能画像を示している。上段(A)および下段(B)に当てはまる薬剤名の組み合わせについて，正しいものを選べ。(第4回　問題15)

a. A; ^{123}I-epidepride　B; ^{123}I-β-CIT
b. A; ^{123}I-β-CIT　B; ^{123}I-IBF
c. A; ^{123}I-iomazenil　B; ^{123}I-IBZM
d. A; ^{11}C-raclopride　B; ^{11}C-SCH23390
e. A; ^{123}I-β-CIT　B; ^{123}I-iomazenil



正解：b

パーキンソン病では，黒質線条体機能ドーパミン神経系の節前機能であるドーパミン合成能の低下とドーパミントランスポーターは低下するが，節後機能であるドーパミンD2受容体は保存される。初期にはup-regulationにより軽度増加している。一方，多系統萎縮症では，ドーパミン合成能，ドーパミントランスポーターの低下に加え，ドーパミン受容体も低下している。上段に対応するものとしては，I-123 β-CITまたはI-123 FP-CIT，下段に対応するものとしては，^{123}I-IBFがある。

問題9　パーキンソン病を示唆する画像所見はどれか。(第5回　問題18)

(1)線条体の血流低下
(2)線条体の^{18}F-DOPAの集積低下
(3)^{123}I-MIBG心筋シンチグラフィの心集積低下
(4)線条体の^{123}I-β-CITの集積低下
(5)線条体の^{123}I-iomazenilの集積低下

正解：(2),(3),(4)

問題10　誤っているのはどれか。2つ選べ。(第7回　問題18)

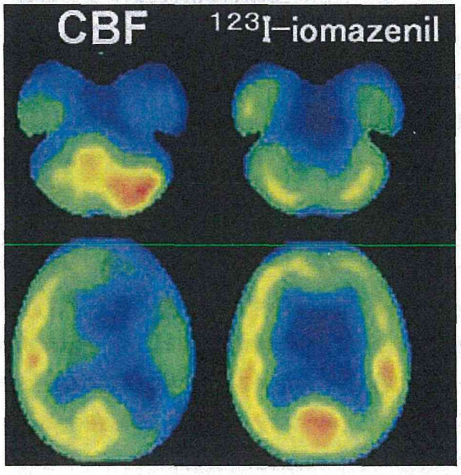
a. ^{123}I-iomazenil —— 中枢性ベンゾジアゼピン受容体
b. ^{11}C-*N*-methylspiperone —— ドーパミンD2受容体
c. ^{11}C-PIB —— Aβアミロイド
d. ^{123}I-IBF —— ノルアドレナリン受容体
e. ^{123}I-β-CIT —— ドーパミンD2受容体

正解：d，e

^{123}I-IBFは，ドーパミンD2受容体イメージング用放射性薬剤である。^{123}I-β-CITはドーパミントランスポーターのイメージングに用いられる。

問題11　症候性左内頸動脈閉塞症に対して行った[I-123]-iodomethamphetamine(IMP)による脳血流(CBF)SPECT画像および[I-123]-iomazenil投与後3時間後SPECT画像を示す。脳血管撮影上他の主幹動脈に狭窄閉塞性病変はなく，MRI上どの部位にも虚血巣は認められない。この画像から考えられる病態のうち，誤っているものを1つ選べ。(第8回　問題14)

a. 左大脳半球の脳血流低下
b. 左大脳半球皮質の神経細胞脱落
c. Crossed cerebellar hypoperfusion
d. 右小脳半球の神経細胞脱落
e. 右大脳半球皮質の貧困灌流



正解：d

I-123 Iomazenil(IMZ)は，中枢性ベンゾジアゼピン系受容体に結合する放射性薬剤である。てんかん焦点において中枢性ベンゾジアゼピン系受容体が低下することから，主としてでてんかん焦点の診断に用いられている。一方，IMZは脳虚血における神経細胞のviabilityの評価用薬剤としても利用できると考えられている。左内頸動脈閉塞に

より，左大脳半球には高度な血流低下と血流ほどではないが，IMZの軽度集積低下を認め，左大脳半球には血流低下とともに細胞脱落もおきていると推測される。右小脳の血流低下もみられ，これは，左大脳半球皮質の細胞障害に伴うcrossed cerebellar diaschisis（CCD）と考えられる。通常，CCDでは経神経的な機能障害のみで小脳皮質の細胞脱落を伴うことはないとされる。本症例でもI-123 IMZ SPECT画像上，小脳半球の左右差はなく，右小脳半球の細胞脱落を示唆する所見はない。

《てんかん》

脳はブドウ糖を唯一のエネルギー源として用いており，脳活動はブドウ糖代謝によって支えられている。従って，神経細胞の過剰放電すなわち活動性の亢進が病気の本質的な部分であるてんかんにおいて，FDG-PETを用いた糖代謝の測定は理に適った検査法といえる。FDG-PET検査は2002年4月より保険診療となり，難治性てんかんにおいて保険適応が認められている。従来はてんかん焦点の同定は，脳波，特に硬膜下電極や海馬深部電極などによりなされていたが，画像診断の進歩により，MRIやPET，SPECTを用いて，焦点と関連する所見が非侵襲的に得られるようになった。特にFDG-PETによる非発作時の糖代謝低下（1），MRIによる海馬容積の低下（2）や海馬硬化を反映したT2強調，FLAIR画像による高信号（3），脳血流SPECTによる発作時の血流増加と非発作時の血流低下（4）などの所見は焦点同定にとって非常に有用な情報である。また，PETやSPECTを用いた受容体などの神経伝達機能測定により，ベンゾジアゼピン系受容体の低下（5）やオピオイド受容体の増加（6）なども報告されている。

てんかんのFDG-PET所見について

FDG-PETは、焦点部検出にもっとも感度が高い検査であり、通常は非発作時検査である。非発作時は焦点およびその近傍は低代謝，低集積となる。まれに発作重積時など比較的長い時間発作が持続する場合には発作を反映した画像が得られ，発作焦点部では代謝亢進，高集積像となる。非発作時の低集積域は，通常焦点部に限局したものではなく，焦点部を含めたより広汎な領域に認められる。内側側頭葉てんかんの場合は，側頭葉内側部だけでなく，外側皮質にも集積低下を認める。また，しばしば同側の視床や線条体にも集積低下を認める。発作時の場合も焦点部のみでなく，焦点部を含めてより広汎に集積増加を認める。一般に，発作時間は短いことが多く，FDGは30分以上かけて徐徐に蓄積していくため，30分以降に撮像されたFDG画像は投与後から撮像時までの平均積算画像となるため，てんかん波の伝播のみならず，発作後の抑制や非発作時の状態が混在した像となることが多い。また大脳の一側に限局した発作では，対側小脳の集積増加を認める（crossed cerebellar activation）こともある。非発作時の糖代謝の低下は，発作の頻度が多く，経過の長い症例ほど検出されることが多い。焦点部はグリオーシス（海馬の場合は海馬硬化）など病理学的変化が背景に存在する可能性が高いが，周辺や遠隔部の糖代謝低下域には，通常，病理学的な変化はなく，あくまでシナプス等の機能的な変化と考えられる。側頭葉てんかんでは海馬扁桃核を中心とした焦点をもつ内側型と側頭葉外側皮質を焦点とする外側型に分けられるが，非発作時FDG-PET検査のみでは，集積低下域はいずれの場合も内側，外側双方に及んでいる場合が多く，区別は困難な場合が多い。また，非発作といっても，検査中に発火がみられる可能性もあり，糖代謝の低下域を検出するという方法のみでは焦点（発火のstarting point）を同定することは困難である。

一般に側頭葉てんかんに比し，前頭葉てんかんなどextra-temporal epilepsyでは発作焦点の検出率は低く，SPMなどの統計画像解析が役立つことも少なくない。

問題12　側頭葉てんかんのSPECT/PET所見に関する記述のうち正しいものを選べ。
（第1回　問題18）

（1）発作時の鎮静剤投与は脳血流に影響を与えない。
（2）側頭葉てんかんの焦点検出率は前頭葉てんかんに比べ高い。
（3）抗てんかん薬の慢性投与で小脳血流が低下する。
（4）^{18}F-FDGの集積低下は^{11}C-flumazenilよりも広い範囲にみられる。
（5）てんかん発作時の高血流域は^{99m}Tc-ECDでは低集積となる。

正解：（2），（3），（4）

問題13　てんかん焦点における以下の検査所見のうち，正しいものはどれか。
（第6回　問題19）
（1）発作間歇期における^{18}F-FDGの集積低下
（2）発作間歇期における^{123}I-β-CITの集積低下
（3）発作期における^{123}I-iomazenilの集積増加
（4）発作期における^{99m}Tc-HMPAOの集積増加
（5）発作間歇期における^{123}I-iomazenilの集積低下
正解：（1），（4），（5）

《脳腫瘍》

脳腫瘍は他の全身の腫瘍同様，一般に悪性度と糖代謝は相関する。ただし，正常神経細胞が糖を盛んに消費するため，必ずしも“hot spot”としては描出されない場合もある。FDG-PETは腫瘍の悪性度を評価し，組織内に悪性のコンポーネントがある程度の大きさで存在することを知るために利用することはできるが，腫瘍の存在範囲，広がりについてはC-11メチオニンが必要な場合も少なくない。Low grade gliomaであるastrocytomaは通常，正常灰白質以下の集積である。一方，悪性のgliomaであるanaplastic astrocytoma（AA）とglioblastoma（GBM）ではほとんどの症例が正常灰白質と同程度ないしそれ以上の集積を示す。したがって，正常灰白質を基準としてそれと同程度以上の集積の場合は悪性神経膠腫の可能生が高いことを常に念頭に置く必要がある。一方，集積が正常白質以下，または同程度の場合はlow grade gliomaの可能性が高い。正常白質と正常灰白質の中間の集積の場合は良性，悪性いずれの可能性もあり慎重な判断が必要となる。しかし，こうしたFDGの集積程度と神経膠腫の病理学的な悪性度との関係に一見，矛盾するような事例もある。元来WHO grade Iと考えられているpilocytic astorocytomaでは，低悪性度に分類されているにもかかわらず，FDGが高集積を呈することが報告されており，グルコーストランスポーターとの関連が推測されている。Anaplastic astrocytoomaとglioblastomaの鑑別については，FDG集積には有意な差はなく，FDG-PET検査でも鑑別はむずかしい。なお，これらの悪性gliomaとの鑑別で重要なものに悪性リンパ腫がある。通常MRIやCTで造影効果を認めるリンパ腫の場合はFDGを高度に取り込むことが多く，正常灰白質よりも際立って集積の高い腫瘍を見た場合は悪性リンパ腫を考慮する必要がある。I-123 IMPによるSPECT検査は，特に後期像で病変部に集積（再分布）がみられ，lymphomaの診断に有用である。

問題14　脳の神経膠腫（glioma）についての記述のうち正しいものを選べ。（第4回　問題5）
a. high grade gliomaとは腫瘍組織の分化度の高い神経膠腫をいう。
b. ^{18}F-FDGが周囲より強く集積する領域として描出される。
c. ^{201}Tl-chlorideの後期像よりも早期像のほうが悪性度をより反映する。
d. 脳血流トレーサの集積程度は腫瘍の血流を反映していない。
e. 再発時には元の腫瘍と同じgradeの組織として再発する。
正解：d

High grade gliomaは，腫瘍組織の分化度の低い神経膠腫を指し，悪性度が高い。神経膠腫再発時は，悪性転化をきたすことが多い。^{201}Tl-chlorideでは後期像のほうが悪性度をより反映する。腫瘍組織の場合，脳血流トレーサの集積程度は，トレーサと組織の親和性の影響を受け，必ずしも血流を反映しない。

《おわりに》

現在，脳神経疾患において最も核医学検査が利用されている領域は「認知症」および関連する神経変性疾患であり，血流・代謝に加え，アミロイドイメージング，ドーパミントランスポーターイメージングなど病理変化や変性を客観的に評価できる核医学検査の果たす役割は今後ますます重要になると思われる。このような分子イメージングとしての特徴は，がんや動脈硬化，神経活動性の異常など様々な疾患領域に展開されていくものと思われる。核医学検査が関わる領域は多岐にわたる。核医学専門医の役割は，適切な検査を施行し，患者様に真に役立つ情報を提供することである。単にコンピューター画面に呈示された画像をみるだけでなく，症状や治療内容を把握し，経過や病理所見を含め，総合的に情報を蓄積し，核医学画像の奥にある患者全体像を観るよう努めなくてはならない。

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 Q_{10}. Levels of coenzyme Q_{10} in lymphoblastoid cells and brain tissue were measured on high-performance liquid chromatography.

RESULTS

We identified a homozygous mutation (M78V-V343A/M78V-V343A) and compound heterozygous mutations (R337X/V343A) in *COQ2* in two multiplex families. Furthermore, we found that a common variant (V343A) and multiple rare variants in *COQ2*, all of which are functionally impaired, are associated with sporadic multiple-system atrophy. The V343A 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.)

The members of the Multiple-System Atrophy Research Collaboration are listed in the Appendix. Address reprint requests to Dr. Shoji Tsuji, Department of Neurology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan, or at tsuji@m.u-tokyo.ac.jp.

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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 nongenetic 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 previously[13]) 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 summarized in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Autopsy findings for Participants II-4[13] 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 software[18] and SAMtools sequence-alignment mapping[19] 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]).

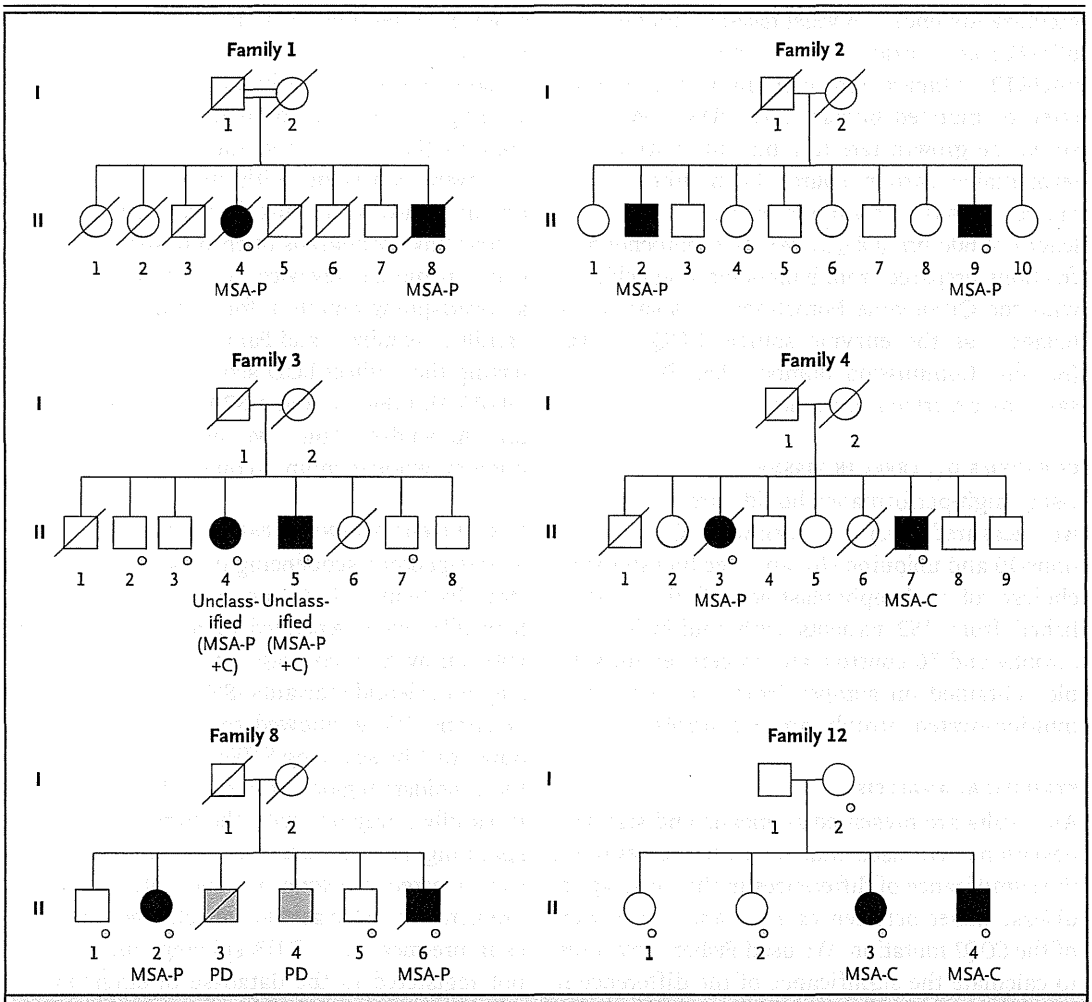

Figure 1. Pedigrees of Six Multiplex Families with Multiple-System Atrophy.

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 M78V-V343A 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 R337X/V343A 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 Q_{10}

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 Q_{10} (*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 BY4741Δ*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 Q_{10} LEVEL IN TISSUES

Using high-performance liquid chromatography, we measured levels of coenzyme Q_{10} (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× 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 in SHROOM3 (Universal Protein Resource [UniProt] accession number, Q8TF72), M78V and V343A in COQ2 (UniProt accession number, Q96H96), and R231G in SCEL (UniProt accession number, O95171).

In the 180 Japanese control samples, we did not observe the SNV encoding the M78V variant but did observe SNVs encoding K706R in SHROOM3, V343A in COQ2, and R231G in SCEL, which were present on 3, 5, and 98 of 360 alleles, respectively. We therefore considered the SNP encoding M78V in *COQ2*, which encodes parahydroxybenzoate-polyprenyl transferase, an enzyme involved in the biosynthesis of coenzyme Q_{10}, 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 members, Participants II-4 and II-8, carried the homozygous M78V-V343A 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 (R337X) and missense (V343A) variants in both affected siblings (Participants II-3 and II-4). Their mother (Participant I-2) was heterozygous for V343A, one unaffected sibling (Participant II-1) lacked this variant, and the other unaffected sibling (Participant II-2) was heterozygous for R337X. R337X 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 Q_{10}, we further sequenced the other 11 genes in the biosynthetic pathway for coenzyme Q_{10} (*PDSS1*, *PDSS2*, *COQ3*, *COQ4*, *COQ5*, *COQ6*, *COQ7*, *ADCK3*, *COQ9*, *COQ10A*, and *COQ10B*) in the remaining four families and in a previously described multiplex family[14] 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, L16V) with allele frequencies of 0.90 and 0.88 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 I97T and a nonmutated [NM] allele at codon 97 and V343A/NM at codon 343, one had R337Q/NM at codon 337 and V343A/NM at codon 343, and two had V343A/V343A), whereas none of the controls had two variants of *COQ2* (Table 1). Sequencing of the subcloned mutated alleles confirmed that R337Q/V343A was present in a compound heterozygous state. We were unable to determine the phase of I97T/V343A, because the distance

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)
P22L/NM	0	1	0	0	0	0
F29L/NM	0	0	1	0	0	0
P49H†/NM	0	0	0	0	1	0
S57T†/NM	0	0	1	0	0	0
R69H†/NM	0	0	0	0	0	1
I97T‡/V343A§	1	0	0	0	0	0
P107S†/NM	1	0	0	0	0	0
S113F†/NM	1	0	0	0	0	0
T267A‡/NM	0	0	1	0	0	0
S297C‡/NM	0	0	1	0	0	0
N336H/NM	0	1	0	0	0	0
R337Q†/V343A§	1	0	0	0	0	0
V343A§/NM	29	17	0	0	0	0
V343A§/V343A§	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* V343A 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)	Comparison with Tier 1		Tier 2 Controls (N=2383)	Comparison with Tier 2	Alzheimer's Disease (N=2728)	Parkinson's Disease (N=659)	ALS (N=634)
			odds ratio (95% CI)	*P value*		*odds ratio (95% CI)*	*P value*			
Allele frequency — no./total no. (%)	35/726 (4.8)	17/1040 (1.6)	3.05 (1.65–5.85)	1.5×10^{-4}	106/4766 (2.2)	2.23 (1.46–3.32)	6.0×10^{-5}	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 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 P49H, S57T, R69H, I97T, P107S, S113F, T267A, S297C, and R337Q (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).

between I97T and V343A was too large to be amplified by means of polymerase-chain-reaction (PCR) assay in a single fragment, and samples of genomic DNA from the parents were unavailable. We found that 29 patients with multiple-system atrophy and 17 controls were heterozygous for the V343A variant. In addition, we detected four novel heterozygous variants: two in patients with multiple-system atrophy (P107S and S113F) and two in controls (P22L and N336H).

Of the *COQ2* variants, the V343A variant is relatively common in the Japanese population. As shown in Table 2, we found that the V343A 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; $P=1.5\times10^{-4}$). Genotyping in the second series of 2383 Japanese controls showed that the V343A variant had an allele frequency of 2.2% (106 of 4766 alleles; odds ratio, 2.23; 95% CI, 1.46 to 3.32; $P=6.0\times10^{-5}$). Genotyping Japanese persons with other neurodegenerative diseases revealed that the V343A 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 V343A variant in patients with multiple-system atrophy. Two patients with Alzheimer's disease who were found to carry homozygous V343A 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 F29L, S57T, T267A, and S297C) among the patients, whereas none of the controls had any variants in *COQ2*. In the North American series, we found one variant (P49H) in a patient with multiple-system atrophy and one variant (R69H) in a control (Table 1). At the time of recruitment for the study, the carrier of R69H, 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 V343A

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 Q_{10} plays an essential

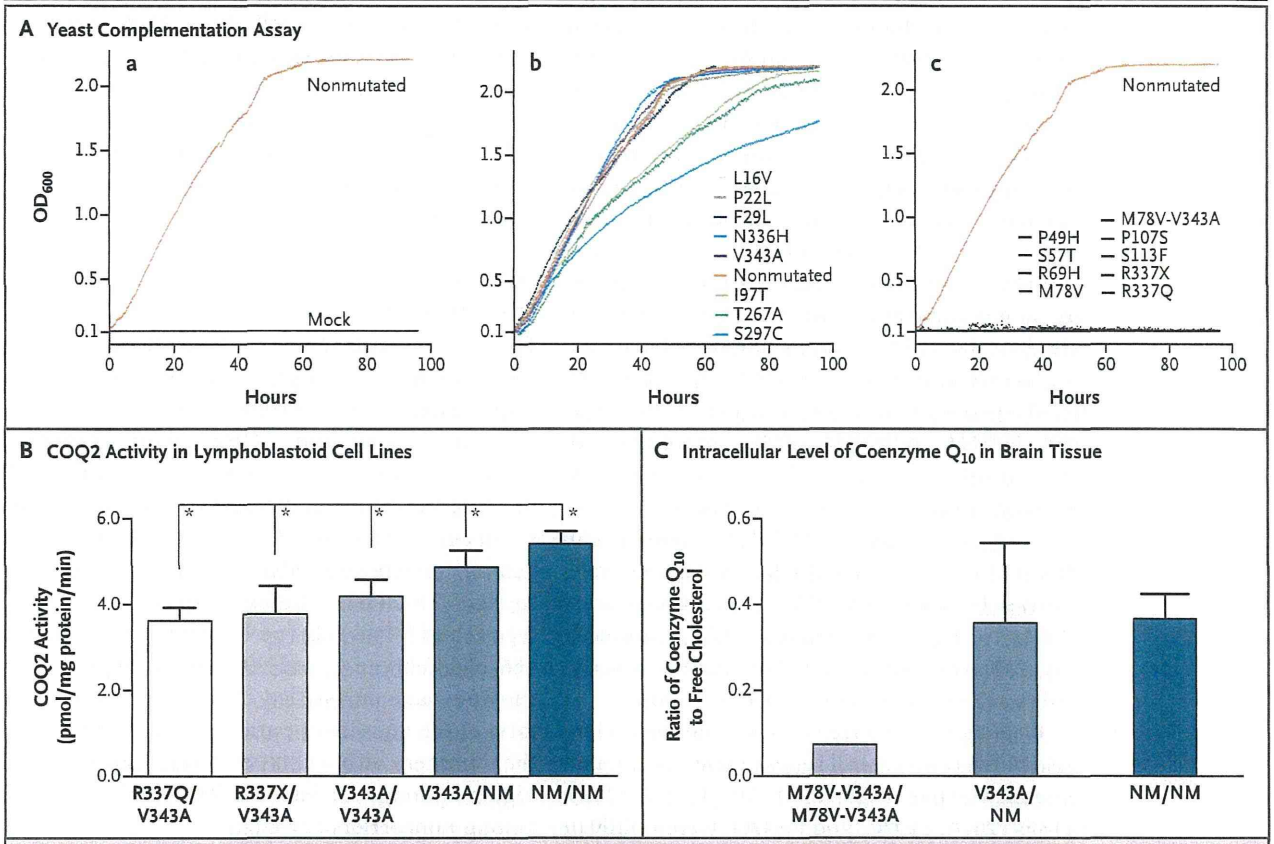

Figure 2. Functional Analysis of *COQ2* Variants.

Panel A shows the results of a yeast complementation assay, including the growth curves of the yeast *coq2*-null mutants transformed with the pAUR123 vector containing the nonmutated (NM) human *COQ2* complementary DNA (cDNA) or mock vector (subpanel a) and those transformed with the AUR123 vector containing different versions of human *COQ2* cDNA (encoding the substitutions L16V, P22L, F29L, N336H, V343A, I97T, T257A, and S297C) (subpanel b). Transformants carrying a *COQ2* allele encoding the I97T, T267A, or S297C substitution show considerably lower growth rates than nonmutated *COQ2*, which still shows a higher growth rate than the *coq2*-null strain (mildly deleterious). Transformants carrying a *COQ2* allele encoding the L16V, P22L, F29L, N336H, or V343A substitution show growth rates similar to that of nonmutated *COQ2*. The *coq2*-null mutants transformed with the pAUR123 vector containing different versions of human *COQ2* cDNA (P49H, S57T, R69H, M78V, M78V-V343A, P107S, S113F, R337X, or R337Q) show severely decreased respiration-dependent growth rates, as does the *coq2*-null strain (severely deleterious) (subpanel c). Each yeast strain was pregrown in yeast extract peptone dextrose (YPD) medium, diluted to an optical density of a sample measured at a wavelength of 600 nm (OD_{600}) of 0.1, and then incubated in yeast extract peptone glycerol (YPG) medium at 23°C with shaking at a rate of 200 rotations per minute for 4 days. OD_{600} was measured every 10 minutes. The experiments were conducted in quadruplicate, and the mean OD_{600} was plotted against the incubation time. Panel B shows COQ2 activity in lymphoblastoid cell lines, as determined by measuring the incorporation of radioactive parahydroxybenzoate (PHB) into decaprenyl-PHB. COQ2 activity was measured in lymphoblastoid cell lines from each patient with multiple-system atrophy carrying *COQ2* variants (encoding R337Q/V343A, R337X/V343A, V343A/V343A, or V343A/NM) and from a control without variants. The mean values from nine independent experiments are shown. T bars indicate standard deviations. Group comparisons were performed with the use of the Kruskal–Wallis test, followed by the Steel test. Asterisks indicate $P<0.05$ for the comparison with a control sample (nonmutated *COQ2* genotype). Panel C shows intracellular levels of coenzyme Q_{10} in frozen cerebellum samples obtained from three patients with multiple-system atrophy (one who was homozygous for M78V-V343A and two who were heterozygous for V343A) and from three controls. Brain-tissue samples weighing approximately 100 mg were homogenized in 10 volumes (volume to weight) of 10 mM TRIS–hydrochloric acid (with a pH of 7.4) containing 0.32 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), and 20% sodium dodecyl sulfate (SDS). Coenzyme Q_{10} was then extracted with hexane and ethanol (in a 5:2 ratio according to volume), and the extract was subjected to high-performance liquid chromatography to measure the level of coenzyme Q_{10}, after adjustment for the free (unesterified) cholesterol level.[21]

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 P49H, S57T, R69H, M78V, M78V-V343A, P107S, S113F, R337Q, and R337X 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 I97T, T267A, and S297C 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 L16V, P22L, F29L, N336H, and V343A alleles, showed growth rates similar to those of the transformants expressing nonmutated *COQ2*. As described above, the yeast strain with M78V-V343A identified in Family 1 showed a severely decreased growth rate, whereas the strain with V343A had a growth rate similar to that of nonmutated *COQ2*, indicating that of the two variants, M78V 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 (P49H, S57T, R69H, I97T, P107S, S113F, T267A, S297C, and R337Q) were mildly or severely deleterious. On combining all three series, eight variants (P49H, S57T, I97T, P107S, S113F, T267A, S297C, and R337Q) were identified in 758 patients with multiple-system atrophy, whereas only one variant (R69H) 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 F29L 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 V343A 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 R337Q/V343A, R337X/V343A, V343A/V343A, or V343A/NM and in a control without variants. The COQ2 activities in the lymphoblastoid cell lines (V343A/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 Q_{10} IN LYMPHOBLASTOID CELL LINES

We measured intracellular coenzyme Q_{10} 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 (R337Q/V343A, R337X/V343A, and V343A/V343A), 16 patients carrying heterozygous V343A, 133 patients without variants, and 76 controls without *COQ2* variants (Table 3). Intracellular levels of coenzyme Q_{10} 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 Q_{10} levels in patients who were heterozygous for V343A and in those without *COQ2* variants were not significantly lower than levels in controls without *COQ2* variants.

Table 3. Intracellular Levels of Coenzyme Q_{10} in Lymphoblastoid Cell Lines, According to *COQ2* Variant.*

Variable	Patients with Multiple-System Atrophy					Controls
	R337Q/V343A	R337X/V343A	V343A/V343A	V343A/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 Q_{10} to free (unesterified) cholesterol reflects the intracellular level of coenzyme Q_{10}. Lower values indicate decreased levels of intracellular coenzyme Q_{10}, presumably reflecting decreased biosynthesis of coenzyme Q_{10}. To calculate the ratio, coenzyme Q_{10} was measured in nanomoles per liter and free cholesterol in micromoles per liter.

‡ Lower values indicate decreased levels of intracellular coenzyme Q_{10}, as compared with the mean value in controls, presumably reflecting decreased biosynthesis of coenzyme Q_{10}.

COENZYME Q_{10} 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 Q_{10} in frozen brain tissues from three patients with *COQ2* variants (one patient who was homozygous for M78V-V343A and two patients with V343A/NM) and from three controls without *COQ2* variants (Fig. 2C). The levels of coenzyme Q_{10} in patients who were homozygous for M78V-V343A 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 (V343A) and multiple rare variants in *COQ2* were associated with sporadic multiple-system atrophy. The V343A variant was found exclusively in the Japanese participants, with an allele frequency of 1.6 to 2.2%. The allele frequency of V343A in patients with multiple-system atrophy (4.8%) was significantly 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 V343A suggests that V343A 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 V343A. 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 V343A 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 population[9] but not in the European population[11] 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 Family 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 Q_{10} 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 Q_{10}.

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 Q_{10} 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 Q_{10} have been well established.[35,36]

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APPENDIX

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