身体所見もかなり改善しました。

笠畑　幻覚を疑わせる病歴や所見はありませんでしたか。

臨床医　明らかな幻覚はないですが，2009年に嚥下性肺炎で入院したときにせん妄状態となり，ミトンなどで抑制をされていました。「側に人が立っている気がする」と，この入院時に訴えています。

笠畑　傾眠など自律神経症状を思わせるような訴えや所見はなかったでしょうか。

臨床医　起立時に意識を失ったとか，傾眠があったとかいうことはありませんでした。

長谷川（横浜市大センター病院）　高血圧はありましたか。

臨床医　血圧は高めですが，内服薬でコントロールせずに130～150 mmHgくらいで推移していました。

長谷川　レボドパを使うくらいのパーキンソン症状があって，それを増量するたびに，それなりには効いているわけですから，ドパミンの欠乏状態はあると思います。しかし，早い時期から嚥下障害や嚥下性肺炎を繰り返しているので，仮性球麻痺の要素が上乗せされていないかどうか，高血圧がもとにあって血管性の病変が加わったという要素はないかと考えお聞きしました。

司会　演題名に「薬剤性パーキンソン症状との鑑別が問題になった」と書いてありますけれども，どうお考えですか。

臨床医　文献には，ソリフェナシン自体がパーキンソン症状を起こす可能性はあるという記載があります。それまで経過のない人が，新しい薬にした直後に，寝返りも打てない，起き上がれないという症状とパーキンソン症状を呈したということから，薬剤性ではないかと疑いました。

ただ，ソリフェナシンをやめてもよくならず，レボドパの内服開始後に改善したことから，潜在していたパーキンソン症状をソリフェナシンが表に出した可能性はあったと思います。そういう点で，当初は鑑別診断として重要だと考えました。

司会　ソリフェナシンがパーキンソン症状を起こすという経験はどれくらいありますか。

仁科（東京都健康長寿医療センター）　私が疑っているのは，2011年度にこの方も含めて2人です。しかし，ソリフェナシンをやめてもよくならないと記憶しています。

コメンテーター（齊藤）　2点確認させていただきます。1点目は，味覚は低下しているが嗅覚の低下はないとあります。これは，嗅覚検査の結果ですか。嗅覚が落ちて料理が下手になるということはなかったのですか。もう1点は，レストレスレッグス症候群ということですけど，非運動症状として出てくるような痛みの訴えはなかったのでしょうか。

臨床医　今回の反省点になりますが，嗅覚検査はしておりません。嗅覚低下はあくまで本人からの訴えです。

レストレスレッグス症候群と診断した根拠は，まず，夕方から夜間にかけて増悪し眠れないと言っていました。そして，動かすと症状が軽快するとも述べています。また，クロナゼパムを飲んで症状がかなり改善したということもあります。

仁科　少し追加しますと，痛みは訴えていません。しびれ感や違和感はあるけれども，「痛い」とは言っていませんでした。

それから，この方は男性で，ご自分で料理するわけではないので，味覚のことに関しては，いわゆる主観的なもの以外はあまりよくわかりません。ここは反省点です。

臨床医（村山）　高齢者で味覚障害を訴える場合には，ほとんど嗅覚障害があると考えています。武田篤先生（東北大学）は，パーキンソン病で複合型嗅覚障害が起きる場合は，3年以内に認知症が発症する確率が高いと報告しておられます。女性の場合には，ご家族に料理の味が変わっていないか――だいたい塩辛くなります――と聞くのですが，男性の場合には「においは何ともない」と言われると，その後が困るんですね。この方の場合にも，そういう意味で確認ができていません。

鈴木（東京慈恵会医科大学葛飾医療センター）　失禁はありましたか。

臨床医　いえ，ありません。

鈴木　通院時は，どういう歩き方をしていたのでしょうか。この年齢ですと，正常圧水頭症もよく合併すると思うのでおうかがいしています。

仁科　この方の歩容は，いわゆる典型的なパーキンソン症状という印象を持っています。例えばすり足とか，少し開脚歩行気味とかいうことはなくて，非常に小歩で，やや前かがみでした。2011年になると歩行器を押して受診されました。

織茂（関東中央病院）　細かいことを3つほどうかがいます。静止時振戦はパーキンソン病によくみられるようなものなのか，左右差があったのかどうか。2つ目は自律神経障害です。具体的に起立性低血圧の検査をされているかどうか。そして3つ目に，中脳被蓋に軽度萎縮ということですけど，中脳と橋の面積比は測っていますでしょうか。

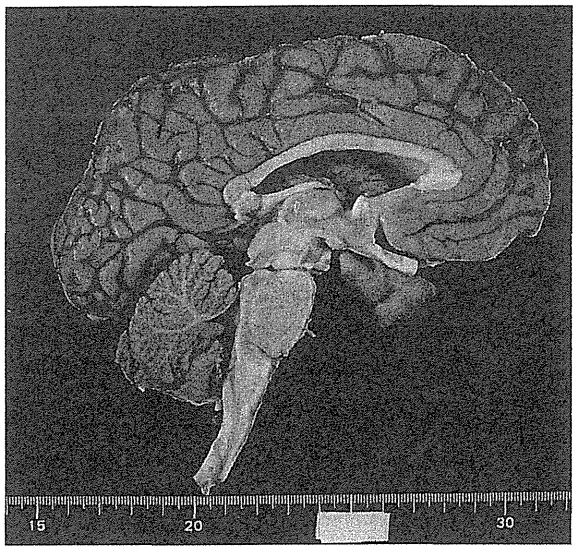

Fig. 1　固定後の左半球内側面
萎縮は前頭極と側頭極に軽度認める。脳幹のボリュームは保たれている。

仁科　この方の静止時振戦はパーキンソン症状として違和感のあるものではなかったと思っています。

臨床医　起立性低血圧に関しては，起き上がったときに意識を失ったというような病歴はありませんでした。実際に検査はしておりません。中脳被蓋萎縮は，面積は下限，橋・中脳被蓋比も同様でした。

臨床医（村山）　われわれの剖検確認症例でも，統計的には，中脳はパーキンソン病では正常とは有意差を持って萎縮します。しかし重なりが大きいので，あまり鑑別上有用性はないと考えています。

後藤（現・済生会横浜市東部病院）　この方の認知機能低下は自覚的なものなのか，周りの人が気づいてきたのは，どんなところなのか，どういう要素が目立ったのかについて教えてください。

仁科　パーキンソン病について何回も説明しているのに，そのときに初めて聞いた，というような対応をすることが多かったという印象があります。一方で「薬を増やしてどうですか」という言い方をすると，それについてはある程度適切な答えが返ってきました。処方した薬に関しては「よくなった」「やめてよくなった」という単純な反応はありました。比較的最近の記憶というのも，それほど落ちていませんでした。むしろ，理解力が落ちていると感じました。

病理所見

司会　では病理に移らせていただきます。パーキンソン症状，軽度の認知症が，どういう病理像に関連しているのかが中心になりますか。

病理医（舟邉）　最初に肉眼所見です。脳重量は1,232 gで，前頭葉と側頭極の萎縮が認められます。動脈硬化は軽度でした。嗅球は両側0.13 gで，当施設で連続採取しているものと比べて，特記すべき重量ではありませんでした。

脳幹を正中で切断した右側の脳幹割面では，橋青斑核は同定が困難です。中脳黒質には脱色素がみられ，正常のものと比較して黒質の色素は薄くなっています。小脳の割面は特に問題ありません。

右半球冠状断では，前方に明らかな梗塞巣などはなく，大脳基底核に大きな梗塞巣などもみられません。脳室の拡大がやや後方に強く認められます。粗大な梗塞巣などはありません。

左半球の内側面と外側面では，前頭葉と側頭極の萎縮を認めます。脳幹のボリュームやバランスは保たれていました（Fig. 1）。左側の脳幹割面では，黒質の色素が薄くなっており，青斑核は正常よりも萎縮していました。小脳の割面に梗塞巣などはありません。

左半球冠状断は，前方には大きな梗塞巣などなく，大脳基底核の萎縮や変性，視床下部の変性など，多系統萎縮症や大脳皮質基底核変性症，進行性核上性麻痺などを疑わせる所見はありません。脳室は後方に強く開大しております。

次に組織学的所見です。

交感神経節のヘマトキシリン・エオジン（HE）染色ではレヴィ小体がみられます。リン酸化αシヌクレインの免疫染色では，細胞質内の封入体，neuritesの陽性所見が認められます。

左室前壁神経束をリン酸化αシヌクレインのモノクローナル抗体とポリクローナル抗体で染めたものでは，neuritesの陽性所見が認められます。リン酸化ニューロフィラメントの免疫染色では，軸索の減少を認め，チロシンヒドロキシラーゼはわずかに陽性です。

皮膚では，好酸性の封入体がみられ，リン酸化αシヌクレインのモノクローナル抗体でもポリクローナル抗体でも，同部位に陽性所見を認めました。副腎では，HE染色ではあまり明瞭ではありませんが，リン酸化αシヌクレイン免疫染色で陽性所見を認めております。胃食道接合部のアウエルバッハ神経叢内にレヴィ小体がみられ，リン酸化αシヌクレイン免疫染色でも陽性となっております（Fig. 2）。

脊髄ですけれども，ほぼ全レベルにおいて前角・後角ともに陽性所見を認め，仙髄では後根内にも陽性所見を

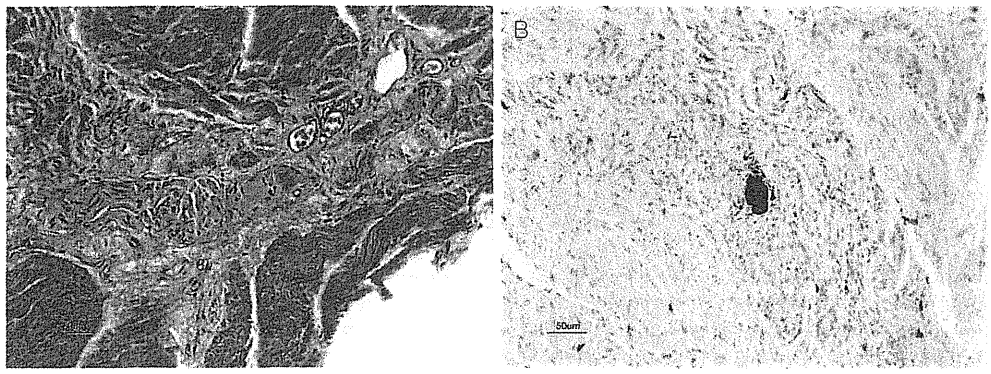

Fig. 2　胃食道接合部
A：ヘマトキシリン・エオジン染色，B：リン酸化 α シヌクレイン免疫染色。バーは 50 μm

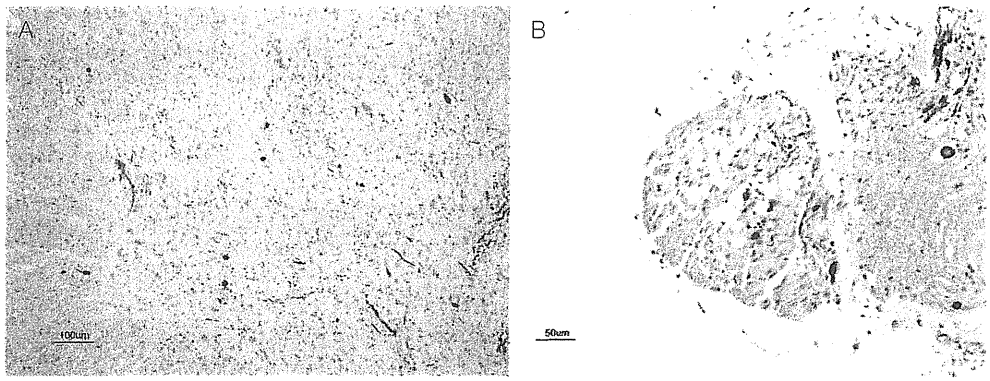

Fig. 3　第 2 仙髄のリン酸化 α シヌクレイン免疫染色
A：前角，B：後角。バーは A が 100 μm，B が 50 μm

認めております（Fig. 3）。胸髄中間外側核には明瞭なレヴィ小体があり，同部位はリン酸化 α シヌクレイン免疫染色で細胞質内の陽性所見と neurites を認めております。

次に脳幹です。延髄迷走神経背側核では，典型的な円状ではなく楕円形で，ハローを伴うレヴィ小体がみられました。神経細胞の脱落があり，リン酸化 α シヌクレイン陽性の細胞質内封入体，neurites, dots を多数認めております。青斑核の神経細胞は著明に脱落しており，リン酸化 α シヌクレイン免疫染色で多数の陽性所見を認めています。

橋縫線核は，HE 染色ではレヴィ小体はあまりはっきりしませんが，免疫染色では多数の陽性所見を認めております。このほか，脚橋被蓋核（pedunculo-pontine nucleus）にハローを伴う明瞭なレヴィ小体がみられ，リン酸化 α シヌクレイン免疫染色でも多数の陽性所見を認めました。中脳黒質の神経細胞は脱落がみられ，HE 染色では遊離メラニンとレヴィ小体を，リン酸化 α シヌクレイン免疫染色でも多数の陽性所見を認めます。

次に嗅覚に関連する所見です。嗅粘膜の中に神経束の陽性所見を認めました。嗅球は，前嗅核に陽性所見を多数認め，辺縁部にも陽性所見を認めています。嗅覚に関連する梨状葉皮質は前頭部・側頭部とも陽性で，側頭部に優位な陽性所見を認めています。

辺縁系では，扁桃核にレヴィ小体が HE 染色でもみられ，リン酸化 α シヌクレイン免疫染色でも陽性所見を多数認めました。海馬においては，CA 2 の神経細胞に細胞質内の陽性所見があり，移行嗅内野にも陽性所見を認めています。前帯状回の皮質，島皮質の両方とも HE 染色でレヴィ小体がみられ，リン酸化 α シヌクレイン免疫染色でも陽性を認めております。大脳基底核では，側坐核，マイネルト基底核，尾状核，被殻と，すべて陽性所見を認めております（Fig. 4）。

大脳皮質では，第 2 前頭回，第 2 側頭回，縁上回にも陽性所見があり，中心前回にもリン酸化 α シヌクレイン陽性の neurites を認めます（Fig. 5）。

以上の結果を DLB コンセンサスガイドラインに当てはめますと，この方は diffuse neocortical に該当する程度のレヴィ小体の広がりということです。

次に梗塞巣ですけれども，左側頭葉の白質に 1 mm

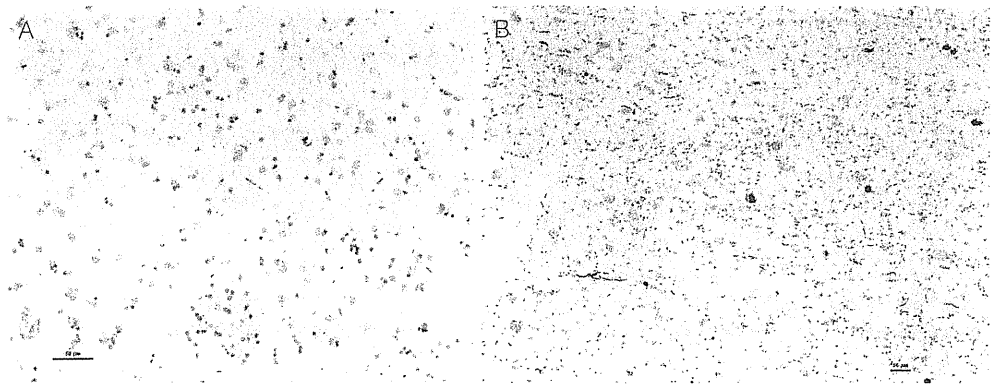

Fig. 4　大脳基底核のリン酸化 α シヌクレイン免疫染色
A：マイネルト基底核，B：尾状核。バーは 50 μm

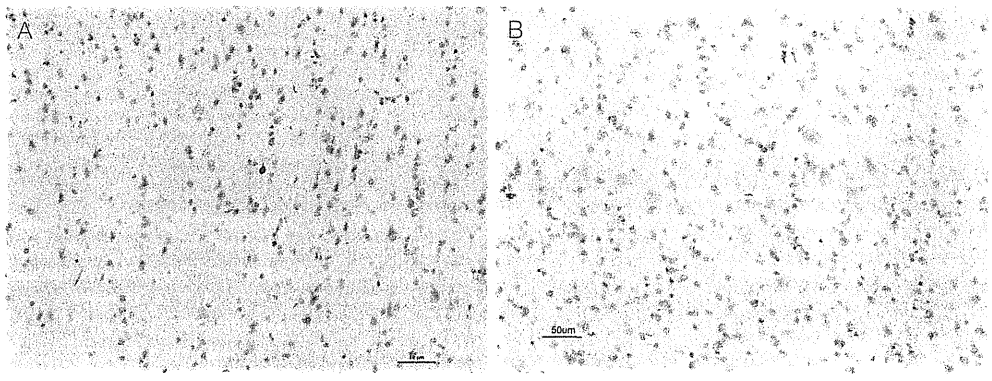

Fig. 5　大脳皮質のリン酸化 α シヌクレイン免疫染色
A：第 2 側頭回，B：中心前回。バーは 50 μm

前後の，顕微鏡でのみ見られるような小さな梗塞巣が認められました。

そのほかの老年性変化ですが，老人斑はごくわずかで，アミロイド β 免疫染色で diffuse plaque が少数皮質に指摘されるのみ，後頭葉でごくわずかに血管壁のアミロイド β 沈着を認めるのみでした。

神経原線維変化は嗅内野，移行嗅内野に中程度認める程度でブラーク（Braak）の NFT ステージ II なので，アルツハイマー病の病理は認知症に関連するものではないと思われます。嗜銀顆粒も確認しましたが，扁桃核ではみられず，リン酸化タウの免疫染色で扁桃核のみに陽性所見を認めたので，今回の病態に関与しないレベルの所見でした。

神経病理診断はレヴィ小体病で，広がりはブラーク分類のステージ VI，他の認知症を説明する病理はなく，軽度認知障害（MCI）を伴うレヴィ小体病と診断されます。

病理診断

1．レヴィ小体病（MCI を伴う）
2．顕微鏡的脳梗塞

ディスカッション 2

司会　画像検査で記載のある大脳基底核の陳旧性梗塞はいかがでしたか。

病理医　少なくとも 7 mm スライスでは，梗塞巣は指摘されませんでした。

司会　薬剤性に関しては病理と合わせて，臨床の先生はどう考えられますか。

横地（荏原病院）　背景にパーキンソン病あるいはレヴィ小体病があり，薬剤は単なるきっかけであったということでいいのではないかと思いました。改めてうかがいたいのは，85 歳まで症状を呈さなかったのはどうしてでしょうか。

病理医　ブラークは，レヴィ小体の広がりは脳幹を上行性に進んで，黒質がやられるとパーキンソン症状が出るといっていますけど，それが大脳皮質に広がるまでに何年かかるかまでは言及しておりません。この所見から3年前はどうであったかを推測するのは難しいですね。

臨床医（村山）　ドパミンPET検査で毎年の低下率は60代の場合，正常では0.5%ぐらい，パーキンソン病の場合には7～8%といわれているのですが，これだけの高齢者のデータはないので，よくわかりません。ただ，本例のように，高齢になればなるほどパーキンソン症状が出た段階でレヴィ小体が全身に広がっているので，パーキンソン病のレボドパの効きも悪いし，いろいろな全身合併症を起こしてくると，私たちは考えています。

高齢者では黒質の黒さは低下していますけれども，50代で発症して70歳近くで亡くなった方のように，黒質が真っ白ということはありません。ですから，黒質でレヴィ小体がみえるというのは，発症してからそんなに時間が経っていないことを意味している気はします。

司会　それでは，齊藤先生にコメントをいただきたいと思います。

コメント

コメンテーター　まず，本例のレヴィ小体病としての位置づけをコメントさせていただきます。レヴィ小体病というのは，レヴィ小体に関連した変性がどこを病変の主座とするかによってパーキンソン病やDLBや純粋自律神経不全症（pure autonomic failure：PAF）というような臨床症状で始まります。しかし，発症してしばらく経って亡くなられてから病理を見てみますと，特に高齢者の場合，臨床情報なしで病理組織標本だけ拝見したら，どの疾患かわからないほど変性が全体に広がっているのが常です。

ただ，多少の強弱はありまして，DLBでは少なくとも嗅球から辺縁系，次いで皮質の病変が強い。パーキンソン病では黒質線条体が強く，PAFでは自律神経系の病変が強いということがあります。

ブラークが最初に報告したのは，レヴィ小体の広がりが脳幹を上行して大脳皮質へ広がるという仮説です。われわれは嗅球を網羅的に検索し，ごく初期には，上行している症例と，嗅球から始まって扁桃核から下，あるいは上にいく症例があるようだと唱えたのですが，本例は，その後ブラークがデュアル・ヒットと呼んだ，下行もするタイプと思われました。

次に薬剤性かどうかです。薬剤をやめたらパーキンソン症状がなくなって，薬剤性と臨床的に診断されていた剖検例の中で，こういった潜在性のDLBの症例は少なくありません。ですから，やはり脆弱性というのはあって，薬剤が症状を出すのを後押ししたのではないかと考えました。

認知障害が疑われますけれども，一般的に責任病巣としては，DLBでもパーキンソン病でも生理学的な最終像は同じです。DLBのコンセンサスガイドラインでは調べるべき領域が第3版で少し増えており，その中にマイネルト基底核と扁桃核が入っております。これは，認知障害に関して重要な場所だと考えられたからです。

マイネルト基底核では，リン酸化αシヌクレイン陽性の突起と神経細胞体内にも陽性所見がありました。扁桃体もかなり重要な役割を占めると思われます。この症例では尾状核の病変はあまり強くなかったようですけれども，高感度の免疫染色でみますと，びまん性の異常所見がありますので，かなり認知障害に関与していると考えています。

レストレスレッグス症候群

それから，レストレスレッグス症候群ということでしたので，調べてみました。特発性と二次性があって，ドパミンの減弱に伴うものが二次性に含まれるのですけれども，頻度は1～50%と，報告にかなり幅があります。よくわかっていないということだと思います。病変部位としてほとんどの神経系が候補に挙がっているのですけれども，私はその中で，視床下部のA11の細胞群というのが大事だと思っています。

今回提示された症例は後角から後根にいくところの病変が強かったので（Fig. 3），これがかなり影響しているのではないかと思いました。

自験例で確認したところ，後角の後根病変がこのように強い症例はあまりありません。私の経験の中では，多い症例でも後角にリン酸化αシヌクレイン免疫染色で陽性所見がある程度です（Table）。今回の症例では，黒質など他の病巣に比べて非常に強い印象がありました。

まとめ

本例は，皮膚，嗅粘膜固有層にも所見がみられるなど，レヴィ小体が脳幹上行進展かつ嗅球扁桃核進展経路をたどって広がっていったと考えました。

軽度認知障害が疑われましたけれども，背景病理としては，CA2の病変に加えて，DLBコンセンサスガイドラインのdiffuse neocortical typeに相当する広がりが

Table　レヴィ小体型認知症におけるレストレスレッグス症候群の背景病理（自験例）

	臨床診断	病理診断	進展経路	前角	後角	後根	後根神経節
89歳，男性	軽度認知障害を伴うパーキンソン病	DLB neocortical	ブラーク上行＋嗅球・扁桃核進展	＋－	＋＋	＋－	－
73歳，女性	パーキンソン病	DLB transitional	ブラーク上行	＋－	＋－	＋－	－
71歳，女性	パーキンソン病	DLB neocortical	ブラーク上行＋嗅球・扁桃核進展	＋－	＋－	－	＋
80歳，女性	認知症を伴うパーキンソン病	DLB neocortical	ブラーク上行＋嗅球・扁桃核進展	＋－	＋	＋	－

〔略語〕DLB neocortical：レヴィ小体型認知症びまん性新皮質型，DBL transitional：レヴィ小体型認知症移行型

関与していたと考えました。

本例から，レヴィ小体病におけるレストレスレッグス症候群の背景病理として，脊髄後角から後根病変も重要な部位の1つと考えられるのではないかと思いました。

ディスカッション3

川田（湘南鎌倉病院）　この症例では嚥下障害がポイントとなると思うのですが，責任病巣としては仮性球麻痺ということでしょうか。

コメンテーター　食道の神経叢にもレヴィ小体が出てきますので（Fig. 2），その関与のほうが大きいのではないかと考えます。

國本（現・済生会神奈川県病院）　病変分布にあった皮膚というのは，いわゆる皮膚固有組織というよりは，皮膚にきている交感神経節後線維ということですか。

コメンテーター　はい，そう考えております。

國本　すべて神経組織内と考えてよろしいでしょうか。

コメンテーター　線維を支配している神経も含んでいます。

司会　皮膚所見をみると，血管の周りの神経軸索や，線維組織周囲の神経突起の中に，α シヌクレイン陽性所見があるようですね。

織茂　レストレスレッグス症候群の背景病理の1つとして後角あるいは後根が関係しているということですけれども，レストレスレッグス症候群は薬でかなりよくなりますね。治療でよくなることと病理所見と，どういう関係にあるのでしょうか。

コメンテーター　後角病変もそうなのですが，視床下部のA11というドパミン作動性のニューロンが大事だということなので，やはり最初はドパミンでしょう。

織茂　それから，今回問題になっている「薬剤性パーキンソン症状」ですけれども，私がよく経験する高齢者の場合，特にドグマチールの50 mg，100 mgの場合，80%以上は背景にレヴィ小体病が隠れているのではないでしょうか。薬をやめても少しパーキンソン症状が残ることはありますけれども，よくなる症例もあります。ただ，そういう患者をみていると，平均1～2年で非常に軽いパーキンソン症状が再び出てくる，ということを経験します。

司会　「薬剤性パーキンソン症状」というのは，詳しく調べていくと非常に面白いということを教えていただきました。どうもありがとうございました。

（症例1・終了）

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Analyses of the *MAPT*, *PGRN*, and *C9orf72* mutations in Japanese patients with FTLD, PSP, and CBS

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ABSTRACT

Background: Mutations in the microtubule associated protein tau (*MAPT*) and progranulin (*PGRN*) have been identified in several neurodegenerative disorders, such as frontotemporal lobar degeneration (FTLD), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS). Recently, *C9orf72* repeat expansion was reported to cause FTLD and amyotrophic lateral sclerosis (ALS). To date, no comprehensive analyses of mutations in these three genes have been performed in Asian populations. The aim of this study was to investigate the genetic and clinical features of Japanese patients with *MAPT*, *PGRN*, or *C9orf72* mutations.
Methods: *MAPT* and *PGRN* were analyzed by direct sequencing and gene dosage assays, and *C9orf72* repeat expansion was analyzed by repeat-primed PCR in 75 (48 familial, 27 sporadic) Japanese patients with FTLD, PSP, or CBS.
Results: We found four *MAPT* mutations in six families, one novel *PGRN* deletion/insertion, and no repeat expansion in *C9orf72*. Intriguingly, we identified a *de novo MAPT* p.S285R mutation. All six patients with early-onset PSP and the abnormal eye movements that are not typical of sporadic PSP had *MAPT* mutations. The gene dosages of *MAPT* and *PGRN* were normal.
Discussion: *MAPT* p.S285R is the first reported *de novo* mutation in a sporadic adult-onset patient. *MAPT* mutation analysis is recommended in both familial and sporadic patients, especially in early-onset PSP patients with these abnormal eye movements. Although *PGRN* and *C9orf72* mutations were rare in this study, the *PGRN* mutation was found in this Asian FTLD. These genes should be studied further to improve the clinicogenetic diagnoses of FTLD, PSP, and CBS.

1. Introduction

Mutations in the microtubule-associated protein tau (*MAPT*) and the progranulin (*PGRN*) genes have been identified in families with frontotemporal dementia and parkinsonism linked to chromosome 17 [1–3]. Recently, two studies reported that the expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene is

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a major cause of both frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) [4,5].

Each of these genes can be associated with multiple clinical entities. Patients with *MAPT* mutations may receive diagnoses of frontotemporal dementia (FTD), primary progressive aphasia (PPA), or progressive supranuclear palsy (PSP). Rarely, corticobasal syndrome (CBS) or FTD with ALS (FTD-ALS) may be manifested in these patients [6]. The clinical diagnoses of patients with *PGRN* mutations include FTD, PPA, and CBS [6]. *C9orf72* repeat expansion causes FTD, ALS, FTD-ALS [4,5], PPA [5,7], and CBS [8] phenotypes. Thus, due to the complicated and often overlapping genetic and phenotypic variability in these patients, an accurate diagnosis of these clinical entities before autopsy is often difficult for clinicians.

To date, few comprehensive screening studies of these three genes have been performed in Asian populations. The aims of this study are to characterize the roles of known and, more importantly, novel disease-causing genes and to investigate the genetic and clinical features of FTLD, PSP, and CBS patients with *MAPT*, *PGRN*, and *C9orf72* mutations. In this study, we also describe the abnormal eye movements that are generally not observed in sporadic PSP but occur in early-onset PSP patients bearing *MAPT* mutations.

2. Methods

2.1. Subjects

We studied 75 Japanese patients who were diagnosed with FTLD, PSP, and CBS with or without a family history of disease. FTLD was divided into three subclasses: behavioral variant FTD (bvFTD), FTD-ALS, and PPA. The clinical diagnoses were established according to the consensus criteria for FTD [9], PPA [10], PSP [11], and CBS [12]. The characteristics of the 75 analyzed patients (69 index patients) are shown in Table 1. This study was approved by the ethics committee of the Juntendo University School of Medicine. Each subject provided written informed consent. All of the subjects in the control cohort were Japanese individuals and were evaluated by neurologists to ensure that no subjects exhibited any clinical manifestations of neurodegenerative diseases.

2.2. Genetic analyses

For direct sequence analysis, each exon was amplified by polymerase chain reaction (PCR) using published primers for *MAPT* [13] and *PGRN* [2] in a standard protocol. Dideoxy cycle sequencing was performed using Big Dye Terminator chemistry (Applied Biosystems, Foster City, CA). These products were loaded into ABI310 and 3130 automated DNA sequence analyzers and analyzed with DNA Sequence Analysis software (Applied Biosystems). To provide a qualitative assessment of the presence of an expanded $(GGGGCC)_n$ hexanucleotide repeat in the *C9orf72* gene, we performed repeat-primed PCR as previously described [4]. The normal repeat number of the GGGGCC hexanucleotide was determined in all of the patients using genotyping primers, as previously described [4]. The PCR products were analyzed on an ABI3130 DNA Analyzer and visualized using Gene Mapper software (Applied Biosystems).

Table 1
The clinical diagnoses and characteristics of 75 patients (69 index patients).

Clinical phenotype	No.	% of total	% of Male	Mean (SD) AAO (range, years)	Familial	Sporadic
FTLD	38	50.7	39.5	57.1 (±12.4), 36–78	21	17
bvFTD	29	38.7	34.5	54.5 (±12.6), 36–78	18	11
FTD-ALS	2	2.7	100	67.5 (±1.5), 66–69	1	1
PPA	7	9.3	42.9	65.0 (±7.4), 58–77	2	5
PSP	25	33.3	68.0	59.8 (±13.0), 40–76	18	7
CBS	12	16.0	33.3	58.4 (±9.52), 40–71	9	3
Total	75	100	48.0	58.2 (±12.3), 36–78	48	27
Index patients	69	92.0	46.4	58.9 (±12.4), 36–78	42	27
Relatives	6	8	66.7	50.3 (±6.6), 44–61	6	0

FTLD = frontotemporal lobar degeneration.
bvFTD = behavioral variant frontotemporal dementia.
FTD-ALS = frontotemporal dementia with amyotrophic lateral sclerosis.
PPA = primary progressive aphasia; PSP = progressive supranuclear palsy.
CBS = corticobasal syndrome; SD = standard deviation; AAO = age at onset.

2.3. Multiplex ligation-dependent probe amplification (MLPA)

To confirm the gene dosages of *MAPT* and *PGRN*, we performed MLPA using the SALSA MLPA P275-B1 MAPT-PGRN kit (MRC-Holland, Amsterdam, The Netherlands). The DNA detection/quantification protocol was provided by the manufacturer. The products were quantified using the ABI3130 Genetic Analyzer and Gene Mapper v3.7 (Applied Biosystems). The kit contains 32 probes, including 13 *MAPT* probes (located in exons 1–13) and 5 *PGRN* probes (located in exons 1, 3, 6, 10, and 12) located within other genes on chromosome 17q21. The MLPA data were analyzed as described previously [14].

2.4. Exon-trapping analysis

To determine whether a novel *MAPT* mutation was pathogenic, we performed an exon-trapping analysis. We used a wild-type construct and constructs containing the novel *MAPT* p.S285R or the IVS10+3 intronic mutation [15]. The *MAPT* sequences included exon 10, 34 nucleotides of the upstream intronic sequence and 85 nucleotides of the downstream intronic sequence. The PCR products were subcloned into the splicing vector pSPL3 (Invitrogen, Carlsbad, CA), and exon trapping was performed as described previously [15].

2.5. Paternity testing

Microsatellite analysis with 10 markers (D2S293, D3S3521, D4S2971, D5S495, D6S16171, D7S2459, D8S1705, D16S430, D18S450, and D20S842) was performed in Patient 1 and his parents to confirm paternity.

2.6. TA cloning

The novel *PGRN* heterozygous deletion/insertion found in this study, *PGRN* p.G338RfsX23 (c.1012_1013delGGinsC), was confirmed by cloning the PCR products into the pCR4-TOPO Vector using the TOPO TA Cloning kit (Invitrogen) and sequencing the two haplotypes of the heterozygote.

3. Results

3.1. Results of MAPT analysis

3.1.1. Genetic and molecular analyses of MAPT

In this study, we identified nine patients with *MAPT* mutations from six families. Four heterozygous missense mutations in *MAPT*, p.L266V, p.N279K, p.N296N, and the novel p.S285R (Supplementary Fig. 1), were identified by direct sequencing. None of the 182 normal Japanese controls included in this study had the *MAPT* p.S285R. In addition, we examined the amino acid sequences of the MAPT protein in other species and found that the site of the p.S285R mutation was highly conserved (see Supplementary Fig. 2). The novel p.S285R mutation in *MAPT* was detected in Patient 1 but not in his parents (Fig. 1A and Supplementary Fig. 1). The parentage of this patient and the DNA authenticity were confirmed using a microsatellite panel (see Supplementary Table 1). These results suggest that p.S285R is a *de novo* mutation. To investigate whether the p.S285R mutation is pathogenic, we performed an exon-trapping analysis. The p.S285R mutation produced a marked increase in the splicing of exon 10 (Fig. 1B) and resulted in the overproduction of tau isoforms that contain 4-repeat tau, such as IVS10+3 [15]. These results indicate that the p.S285R mutation is a novel, *de novo* pathogenic mutation. Previously, p.L266V, p.N279K, and p.N296N had been reported as pathogenic mutations [16–18].

Table 2 lists the clinical features of all of the *MAPT*- and *PGRN*-positive patients in this study, and Supplementary Fig. 3 shows Pedigrees C, D, E, F, and G. The average age at disease onset of patients with a single heterozygous *MAPT* mutation was 42.3 ± 2.9 (range: 37–46) years. MLPA analysis showed no gene dosage abnormalities (multiplications or deletions) in *MAPT* in this cohort.

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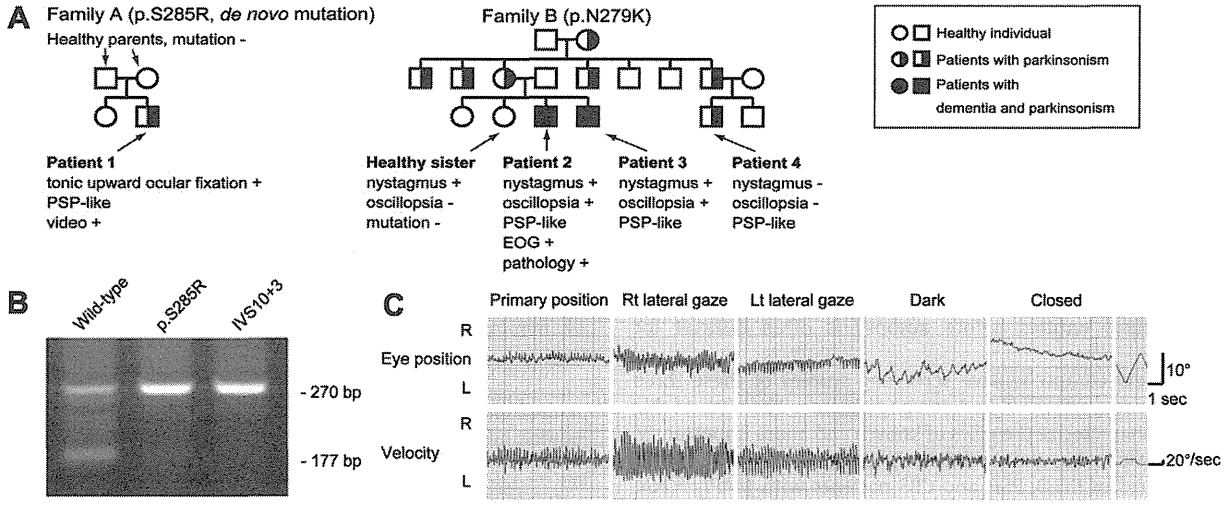

Fig. 1. (A) The pedigrees of families A and B. (B) Exon-trapping analysis for the effects of the *MAPT* p.S285R mutation on exon 10 splicing. (C) Horizontal electro-oculogram recordings in Patient 2.

3.1.2. Clinical presentations of MAPT-positive patients with the abnormal eye movements that are generally not observed in patients with sporadic PSP

3.1.2.1. Patient 1 (MAPT p.S285R). This patient was a 46-year-old man who presented with difficulty speaking and breathing. The patient had no family history of dementia or movement disorders (Fig. 1A). A physical examination revealed gait disturbance, limb bradykinesia, and frequent falling. At age 47, the patient exhibited palilalia and a mild obsession with eating. The patient's Mini-Mental State Examination (MMSE) score was 28/30, but his Frontal Assessment Battery score was 12/18. The patient exhibited a slowing of saccadic eye movements with a relative preservation of smooth pursuit, vertical supranuclear gaze palsy, and tonic upward ocular fixation (see Video Supplement); when the patient's eyes opened after closing, they remained fixated upward and could not be moved voluntarily to the primary position (i.e., Bell's phenomenon remained). To overcome this disability, the patient extended his neck, which resulted in a reflex downward movement of the eyes (the vestibulo-ocular reflex), and next he slightly flexed his neck to a neutral position with his eyes in the primary position. Later, the patient developed bradykinesia and postural instability with frequent falling. L-dopa/benserazide (up to 900 mg/day) was ineffective. The patient's condition gradually deteriorated, and he developed dementia, retrocollis, vertical and horizontal supranuclear palsy, and bradykinesia. At age 49, the patient died of suffocation from the aspiration of food material. No autopsy was performed. The clinical diagnosis was probable PSP.

3.1.2.2. Patient 2 (MAPT p.N279K). This patient was the older brother of Patient 3 (Fig. 1A). Patient 2 was a 42-year-old man who exhibited oscillopsia, micrographia, and a shuffling gait. This patient reported having had nystagmus without oscillopsia since childhood. A neurological examination revealed marked horizontal nystagmus. The patient's pupils were isocoric, and his visual acuity was normal. The patient presented with rigidity, bradykinesia, and postural tremor in the upper limbs. Electro-oculography revealed horizontal pendular nystagmus in the primary position and in all gaze directions (Fig. 1C). L-dopa/benserazide at 200 mg/day mildly alleviated his parkinsonism. Two years later, the patient developed prominent postural instability and became prone to falling. Upward and downward gaze palsy and apraxia of eyelid opening were also noted. At that time, the clinical diagnosis was possible PSP with a family history of dementia and parkinsonism. The patient's cognitive function deteriorated gradually. At age 52, he was bedridden and required a gastrostomy. The patient died of pneumonia at age 54. A postmortem pathological examination of the brain revealed mild atrophy of the frontal lobe and the tegmentum of the midbrain and pons. Microscopic analysis showed severe degenerative changes in the substantia nigra and the subcortical nuclei. Immunohistochemistry using anti-phosphorylated tau (p-tau) antibodies revealed numerous tau-positive neuronal and glial inclusions in the frontotemporal cortex, white matter, and the subcortical nuclei (see Supplementary Fig. 4). These p-tau deposits reacted with anti-4-repeat tau antibodies but not with anti-3-repeat tau antibodies.

3.1.2.3. Patient 3 (MAPT p.N279K). This patient was the younger brother of Patient 2 (Fig. 1A). At age 44, Patient 3 noticed clumsiness in his right hand and oscillopsia. The patient reported having nystagmus since childhood. A neurological examination revealed large, horizontal pendular nystagmus in the primary position and in all gaze directions. The patient's visual acuity, pupils, and light reflexes were all normal. Mild bradykinesia and rigidity in the neck and the right upper limb were noted. Postural tremor in both hands and the tongue and postural instability were observed. Treatment with 600 mg/day of L-dopa/carbidopa was not effective. The patient's oscillopsia gradually worsened, and eventually he was unable to read printed materials. At age 47, the patient developed upward and downward gaze palsy, slowing of saccades, and apraxia of eyelid opening. The patient had prominent postural instability and was prone to falling. The patient's first clinical diagnosis was possible PSP with a family history of dementia and parkinsonism. The patient died at age 56. An autopsy was not performed.

3.1.2.4. Patients 5, 6, and 7 (MAPT p.N279K). The clinical presentations of these three patients have been described previously [19]. All three patients had clinical diagnoses of possible PSP (Table 2) and visual grasping [19,20].

3.2. Results of PGRN analysis

3.2.1. Genetic Analyses of PGRN

We identified one patient with a *PGRN* mutation (Table 2, Supplementary Fig. 3). One novel heterozygous deletion/insertion

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Table 2
Clinical features of patients with *MAPT* and *PGRN* mutations.

Family	A	B			C	D		E	F	G
Patient	1	2	3	4	5	6	7	8	9	10
Gene					*MAPT*					*PGRN*
Genotyping					Heterozygous					
Nucleotide change	c.853A > C	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.837T > G	c.796C > G	c.888T > C	c.1012_1013delGGinsC
Amino acid change	p.S285R	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.N279K	p.L266V	p.N296N	p.G338RfsX23
Exon	10	10	10	10	10	10	10	9	10	9
Mode of inheritance	*de novo*	AD	AD	AD	NA	AD	AD	AD	AD	AD
Age at onset, years	46	42	44	46	41	42	43	37	44	59
Age at evaluation, years	47	47	45	50	44	44	45	38	49	61
Age at death, years	49	54	56	alive	51	54	51	alive	alive	alive
Sex	M	M	M	M	F	F	F	F	M	F
Clinical syndromes	PSP	PSP	PSP	PSP	PSP	PSP	PSP	bvFTD	PSP	PPA
Clinical features										
Initial symptoms	P	P	P	P	P	P	P	dementia	P	aphasia
Personality/behavior changes	−	+	−	−	−	−	−	+	+	−
Mini mental state examination score	28/30	NA	NA	28/30	NA	NA	NA	0	24/30	29/30
Hasegawa dementia scale-revised[a]	NA	18/30	NA	NA	21/30	28/30	30/30	0	21/30	29/30
Nonfluent spontaneous speech	−	−	−	−	−	−	−	−	−	+
Apraxia of eyelid opening	−	+	+	+	+	+	+	−	−	−
Abnormal eye movements										
Supranuclear gaze palsy	+	+	+	+	+	+	+	−	+	−
Tonic upward ocular fixation	+	−	−	−	−	−	−	−	−	−
Oscillopsia with CN	−	+	+	−	−	−	−	−	−	−
Visual grasping	−	−	−	−	+	+	+	−	−	−
Parkinsonism										
Bradykinesia	+	+	+	+	+	+	+	−	+	−
Rigidity	−	+	+	+	+	+	+	−	+	−
Tremor	−	+	+	−	−	−	−	−	−	−
Postural instability	+	+	+	+	+	+	+	−	+	−
Response to L-dopa	−	partial[b]	−	partial[b]	partial[b]	partial[b]	partial[b]	NA	+	NA
Pyramidal sign	+	−	NA	−	+	−	+	+	+	−
Features of motor neuron disease	−	−	−	−	−	−	−	−	−	−
Reference					[19]	[19]	[19]			

AD = autosomal dominant.
P = parkinsonism; NA = not available.
CN = congenital nystagmus; PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia; PPA = primary progressive aphasia.
[a] The Hasegawa dementia scale-revised is a brief dementia screening scale. The maximum score of the Hasegawa dementia scale-revised is 30 points. There was a significant difference in the mean score between the demented and non-demented subjects when the cut-off point was set at 20/21 [31].
[b] A partial response to L-dopa indicates that L-dopa was effective only in the early stages.

mutation in *PGRN*, p.G338RfsX23 (c.1012_1013delGGinsC), was detected by direct sequencing and TOPO TA cloning sequencing (Supplementary Fig. 1). None of the 182 normal Japanese controls included in this study had the *PGRN* p.G338RfsX23 (c.1012_1013delGGinsC) mutations. The age at disease onset of the patient with the heterozygous *PGRN* deletion/insertion was 59 years. Novel *PGRN* variants with unknown significance, p.R18Q and p.N118del, are listed in Table 3. MLPA analysis showed no gene dosage abnormalities in *PGRN*.

3.2.2. A clinical presentation of a novel PGRN mutation

3.2.2.1. Patient 10 (PGRN p.G338RfsX23, c.1012_1013delGGinsC). This patient, a 59-year-old woman, developed word-finding difficulties and underwent surgical clipping at age 54 for an unruptured

Table 3
Novel variants with unknown significance.

Gene	Nucleotidechange	Amino acid change	Exon	Amino acid conservation	Mean AAO (years)	Frequency		*P* value	Clinical diagnosis
						Patients *N* (%)	Controls *N* (%)		
PGRN	c.56G > A	p.R19Q	1	not conserved	66	1/69 (1.4)	0/186 (0)	0.605	PSP ($n = 1$)
PGRN	c.352_354delAAC	p.N118del	4	not conserved	53	3/69 (4.3)	3/272 (1.1)	0.187	bvFTD ($n = 3$)

AAO = age at onset.
PSP = progressive supranuclear palsy.
bvFTD = behavioral variant frontotemporal dementia.

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aneurysm of the left middle cerebral artery. The patient's mother suffered from dementia, but the details of her disease were unknown. The patient substituted words for names of people and objects. Two years after the onset of symptoms, the patient became severely disfluent. However, she did not show any violent behavior, personality changes, or other behavioral abnormalities. The patient scored 29/30 on the MMSE. On the frontal assessment battery, she scored 13/18. The patient's time to complete the Trail Making Test (TMT) A was 70 s, and she could not finish the TMT B within five minutes. Her spontaneous speech production was characterized by slow and hesitant speech, frequently interrupted by long word-finding pauses. Her motor speech abilities were within the normal limits, and no apraxia of speech was noted. No parkinsonism was observed. The patient's clinical diagnosis was PPA with a family history of dementia.

3.3. Results of C9orf72 analysis

We identified no patients with expanded hexanucleotide repeats in *C9orf72* in this study. In 75 patients, the average repeat number based on fluorescent fragment-length analysis was 3.77 ± 2.56 (range 2–11 repeats). We have previously reported that an analysis of 197 Japanese healthy controls did not find any *C9orf72* mutation. The average repeat number was 3.69 ± 2.46 (range 2–14 repeats) in the 197 controls [21].

4. Discussion

We identified five *MAPT* mutations, including a novel *de novo* mutation and a novel *PGRN* mutation, and we found no *C9orf72* mutations in our 75 patients. More mutations were found in *MAPT* than in the other two genes evaluated in this study. The infrequent observation of *PGRN* and *C9orf72* mutations might be partly due to the small number of FTLD patients included ($n = 38$) because the majority of *PGRN* and *C9orf72* mutations have been described in patients with FTLD. In contrast to most other mutation screening studies, we performed MLPA analysis to ensure that exonic or larger deletions or multiplications of *MAPT* and *PGRN* would be identified. Therefore, our data also show that multiplications of *MAPT* and exonic or genomic deletions in *PGRN* are rare in Asian populations. Although mutations were detected in FTLD and PSP patients, we did not find any mutations in our CBS patients. A further larger study and investigation of the other genes are needed to clarify the genetic background of Japanese patients with CBS.

The *MAPT* p.S285R mutation, which we found in this study, is a novel *de novo* mutation. To the best of our knowledge, this report is the first description of an adult sporadic case of a *de novo MAPT* mutation associated with dementia and parkinsonism. All six patients (Patients 1, 2, 3, 5, 6, and 7) with PSP and the distinct eye movements described in the present study (such as tonic upward ocular fixation, oscillopsia with congenital nystagmus, and visual grasping) harbored *MAPT* mutations. Below, we discuss these abnormal eye movements, which are generally not observed in patients with sporadic PSP.

In Patient 1 (*MAPT* p.S285R), we observed tonic upward ocular fixation, which is a loss of downward saccades resembling an acquired ocular motor apraxia [22]. This condition is characterized by a loss of voluntary control of saccades and pursuit, whereas reflex movements—in particular, the vestibulo-ocular reflex—were preserved. Acquired ocular motor apraxia is usually the result of bilateral frontal or frontoparietal infarcts. Therefore, tonic upward ocular fixation due to a *MAPT* mutation might share "supranuclear" cerebral lesions in common with ocular motor apraxia. Brainstem functions, including the vestibulo-ocular reflex and Bell's phenomenon, were preserved in Patient 1.

In Patients 2 and 3 (*MAPT* p.N279K), pendular nystagmus was present since childhood and was suppressed with eyelid closure. These features are consistent with congenital nystagmus [23]. Most patients with congenital nystagmus do not complain of oscillopsia, despite having nearly continuous eye movement [23]. Notably, Patients 2 and 3 noticed oscillopsia when they developed parkinsonism. In these siblings, cerebral lesions caused solely by a *MAPT* mutation were unlikely to be the cause of their nystagmus; however, the co-existence of congenital nystagmus and the *MAPT* mutation might have caused the oscillopsia. This notion is supported in part because the patients had a sister who remained healthy – even in her late 60s – and did not complain of oscillopsia, despite having obvious pendular nystagmus (Fig. 1A). Thus, *MAPT* mutations might impair the visual-motion processing pathways that would normally suppress oscillopsia in patients with common congenital nystagmus. Visual grasping, which was first described by Ghika et al. [20], was observed in Patients 5, 6, and 7 (*MAPT* p.N279K) [19].

Although PSP is a rare manifestation of *MAPT* mutation [24], and the routine screening of sporadic PSP for mutations in *MAPT* is not recommended because of low yield [25], it is recommended that screening be considered for families in which there is an autosomal dominant history of a PSP syndrome, particularly when there are accompanying features suggestive of bvFTD [24]. The clinical difference from sporadic PSP might sometimes be difficult to detect, especially in patients without a family history [26–28]; however, an important case report indicated that an age at disease onset under 50 years combined with the absence of early falling may indicate a possible *MAPT* mutation in clinically diagnosed PSP, even in the absence of a positive family history [26]. Consistent with this observation, our eight *MAPT*-positive patients with PSP phenotype were younger than 50 years at disease onset (Table 2). We further suggest that it may be useful to test for *MAPT* mutations in early-onset PSP patients with the abnormal eye movements that are not typical of sporadic PSP. In fact, we identified the novel *de novo* mutation p.S285R in Patient 1 and p.N279K in Patient 5, who had no family history, after focusing on these clinical phenotypes.

To the best of our knowledge, the *PGRN* mutation has not been previously described in Asian populations [29]. We detected a novel *PGRN* mutation, p.G338RfsX23 (c.1012_1013delGGinsC), and thus showed that *PGRN* mutations may exist in Asian populations. This mutation introduces a premature termination codon at the same site as the p.G333VfsX28 (c.998delG) mutation, which was reported previously, and produced a PPA phenotype in all of the affected individuals [30]. The PPA phenotype of p.G338RfsX23 (c.1012_1013delGGinsC) in our study is remarkably similar to that of p.G333VfsX28 (c.998delG), especially in the manifestation of word-finding and object-naming difficulties and the lack of memory or personality changes during the first few years after symptom onset. We believe that the mutant RNA in both cases is most likely subjected to nonsense-mediated decay, similar to other *PGRN* mutations [2].

In summary, based on these findings, we recommend genetic testing for *MAPT* mutations not only in familial patients but also in sporadic patients, especially early-onset PSP patients with the abnormal eye movements that are generally not observed in sporadic PSP. Although *PGRN* and *C9orf72* mutations were rare in this study, we determined that the *PGRN* mutation does exist in Asian patients with FTLD (PPA). Based on the clinical information, screening for *MAPT*, *PGRN*, and *C9orf72* mutations should be further undertaken to improve the diagnosis of specific clinical entities of neurodegenerative disorders.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.parkreldis.2012.06.019.

References

[1] Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702–5.
[2] Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006;442:916–9.
[3] Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006;442:920–4.
[4] Dejesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72:245–56.
[5] Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-Linked ALS-FTD. Neuron 2011;72:257–68.
[6] Boeve BF, Hutton M. Refining frontotemporal dementia with parkinsonism linked to chromosome 17: introducing FTDP-17 (MAPT) and FTDP-17 (PGRN). Arch Neurol 2008;65:460–4.
[7] Murray ME, Dejesus-Hernandez M, Rutherford NJ, Baker M, Duara R, Graff-Radford NR, et al. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. Acta Neuropathol 2011;122:673–90.
[8] Lindquist S, Duno M, Batbayli M, Puschmann A, Braendgaard H, Mardosiene S, et al. Corticobasal and ataxia syndromes widen the spectrumof C9ORF72 hexanucleotide expansion disease. Clin Genetics 2012 May 31 [Epub ahead of print].
[9] Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546–54.
[10] Mesulam MM. Slowly progressive aphasia without generalized dementia. Ann Neurol 1982;11:592–8.
[11] Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 1996;47:1–9.
[12] Boeve BF, Lang AE, Litvan I. Corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. Ann Neurol 2003;54(Suppl. 5):S15–9.
[13] Baker M, Kwok JB, Kucera S, Crook R, Farrer M, Houlden H, et al. Localization of frontotemporal dementia with parkinsonism in an Australian kindred to chromosome 17q21-22. Ann Neurol 1997;42:794–8.
[14] Keyser RJ, Lombard D, Veikondis R, Carr J, Bardien S. Analysis of exon dosage using MLPA in South African Parkinson's disease patients. Neurogenetics 2010;11:305–12.
[15] Varani L, Hasegawa M, Spillantini MG, Smith MJ, Murrell JR, Ghetti B, et al. Structure of tau exon 10 splicing regulatory element RNA and destabilization by mutations of frontotemporal dementia and parkinsonism linked to chromosome 17. Proc Natl Acad Sci U S A 1999;96:8229–34.
[16] Kobayashi T, Ota S, Tanaka K, Ito Y, Hasegawa M, Umeda Y, et al. A novel L266V mutation of the tau gene causes frontotemporal dementia with a unique tau pathology. Ann Neurol 2003;53:133–7.
[17] Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, et al. Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. Proc Natl Acad Sci U S A 1998;95:13103–7.
[18] Spillantini MG, Yoshida H, Rizzini C, Lantos PL, Khan N, Rossor MN, et al. A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. Ann Neurol 2000;48:939–43.
[19] Ogaki K, Motoi Y, Li Y, Tomiyama H, Shimizu N, Takanashi M, et al. Visual grasping in frontotemporal dementia and parkinsonism linked to chromosome 17 (microtubule-associated with protein tau): a comparison of N-Isopropyl-p-[(123)I]-iodoamphetamine brain perfusion single photon emission computed tomography analysis with progressive supranuclear palsy. Mov Disord 2011;26:561–3.
[20] Ghika J, Tennis M, Growdon J, Hoffman E, Johnson K. Environment-driven responses in progressive supranuclear palsy. J Neurol Sci 1995;130:104–11.
[21] Ogaki K, Li Y, Atsuta N, Tomiyama H, Funayama M, Watanabe H, et al. Analysis of C9orf72 repeat expansion in 563 Japanese patients with ALS. Neurobiol Aging 2012 June 21 [Epub ahead of print].
[22] Pierrot-Deseilligny C, Gautier JC, Loron P. Acquired ocular motor apraxia due to bilateral frontoparietal infarcts. Ann Neurol 1988;23:199–202.
[23] Leigh RJZD. In: The neurology of eye movements. 4 ed. New York: Oxford University Press; 2006. p. 512–21, 638–45.
[24] Rohrer JD, Paviour D, Vandrovcova J, Hodges J, de Silva R, Rossor MN. Novel L284R MAPT mutation in a family with an autosomal dominant progressive supranuclear palsy syndrome. Neurodegener Dis 2011;8:149–52.
[25] Williams DR, Pittman AM, Revesz T, Lees AJ, de Silva R. Genetic variation at the tau locus and clinical syndromes associated with progressive supranuclear palsy. Mov Disord 2007;22:895–7.
[26] Morris HR, Osaki Y, Holton J, Lees AJ, Wood NW, Revesz T, et al. Tau exon 10 + 16 mutation FTDP-17 presenting clinically as sporadic young onset PSP. Neurology 2003;61:102–4.
[27] Rossi G, Gasparoli E, Pasquali C, Di Fede G, Testa D, Albanese A, et al. Progressive supranuclear palsy and Parkinson's disease in a family with a new mutation in the tau gene. Ann Neurol 2004;55:448.
[28] Ros R, Thobois S, Streichenberger N, Kopp N, Sanchez MP, Perez M, et al. A new mutation of the tau gene, G303V, in early-onset familial progressive supranuclear palsy. Arch Neurol 2005;62:1444–50.
[29] Kim HJ, Jeon BS, Yun JY, Seong MW, Park SS, Lee JY. Screening for MAPT and PGRN mutations in Korean patients with PSP/CBS/FTD. Parkinsonism Relat Disord 2010;16:305–6.
[30] Mesulam MM. Primary progressive aphasia—a language-based dementia. N Engl J Med 2003;349:1535–42.
[31] Imai Y, Hasegawa K. The revised hasegawa's dementia scale (HDS-R) – evaluation of its usefulness as a screening test for dementia. J Hong Kong Coll Psychiatr 1994;4:20–4.

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RESEARCH ARTICLE

VPS35 Mutation in Japanese Patients with Typical Parkinson's Disease

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ABSTRACT: Vacuolar protein sorting 35 *(VPS35)* was recently reported to be a pathogenic gene for late-onset autosomal dominant Parkinson's disease (PD), using exome sequencing. To date, *VPS35* mutations have been detected only in whites with PD. The aim of the present study was to determine the incidence and clinical features of Asian PD patients with *VPS35* mutations. We screened 7 reported nonsynonymous missense variants of *VPS35*, including p.D620N, known as potentially disease-associated variants of PD, in 300 Japanese index patients with autosomal dominant PD and 433 patients with sporadic PD (SPD) by direct sequencing or high-resolution melting (HRM) analysis. In addition, we screened 579 controls for the p.D620N mutation by HRM analysis. The p.D620N mutation was detected in 3 patients with autosomal dominant PD (1.0%), in 1 patient with SPD (0.23%), and in no controls. None of the other reported variants of *VPS35* were detected. Haplotype analysis suggested at least 3 independent founders for Japanese patients with p.D620N mutation. Patients with the *VPS35* mutation showed typical tremor-predominant PD. We report Asian PD patients with the *VPS35* mutation. Although *VPS35* mutations are uncommon in PD, the frequency of such mutation is relatively higher in Japanese than reported in other populations. In *VPS35*, p.D620N substitution may be a mutational hot spot across different ethnic populations. Based on the clinical features, *VPS35* should be analyzed in patients with PD, especially autosomal dominant PD or tremor-predominant PD.

Key Words: Parkinson's disease; *VPS35*; autosomal dominant; hotspot; mutation.

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive motor disturbances manifested by tremor, rigidity, akinesia, and postural instability. Neuropathologically, PD is characterized by selective loss of dopaminergic neurons in the substantia nigra and the presence of cytosolic inclusions called Lewy bodies (LBs) in the remaining neurons. The pathogenesis of PD is multifactorial, including genetic–environmental interaction. PD is a common disease in the elderly, with an incidence of about 1%–2% in individuals older than 60 years.[1] Among PD patients, approximately 5%–10% have a positive family history of PD,[2] and among these, the Mendelian forms of PD can contribute to the elucidation of the molecular pathways that lead to the degeneration and death of dopaminergic neurons.

Mutations in the vacuolar protein sorting 35 (*VPS35*) gene have recently been identified in families with autosomal dominant late-onset PD (MIM 601501).[3,4] Patients with *VPS35* mutations present with tremor-predominant dopa-responsive parkinsonism.[3,4] VPS35, a key component of the retromer cargo-recognition complex, is thought to associate with sorting cargos into the tubular endosomal network for retrieval to the trans-Golgi network.[5] Therefore, pathogenic mutations of *VPS35* may cause disruption of the retrograde transport system and contribute to dopaminergic neuronal cell death in PD. One missense mutation has been reported to be pathogenic for PD.[3,4] Mutation of c.1858G>A (p.D620N) was identified in 3 Austrian families and 1 family each in Switzerland, the United States, Tunisia, and the United Kingdom, as well as 1 family and 1 patient with sporadic PD (SPD) among Yemenite Jews from Israel.[3,4,6] In addition, several variants, such as p.M57I, p.I241M, p.P316S, and p.R524W, have been reported in Europe and the United States as potentially pathogenic for PD.[3,4]

Although multipopulation screenings for *VPS35* mutations were preformed in recent reports, there is still no report of PD patients with *VPS35* mutations of Asian ancestry.[3,4,6–8] In the present study, we screened Japanese patients with autosomal-dominant PD (ADPD), Japanese patients with SPD, and control subjects for mutations of *VPS35*, with a special focus on 7 reported nonsynonymous variants that were found in patients with PD, including the p.D620N. Here, we report 3 families and 1 SPD patient with the p.D620N mutation in *VPS35* and describe their clinical features.

Patients and Methods

Subjects

The study was approved by the ethics committee of Juntendo University, and all subjects gave written informed consent to participate in the genetic research. The study subjects were 308 Japanese patients (300 index patients) with ADPD (age at disease onset [AAO; mean ± SD], 51.1 ± 11.7 years; range, 8–83 years; female/male [F/M] ratio, 1.35) and 433 Japanese SPD patients (AAO, 47.2 ± 12.9 years; range, 5–88 years; F/M ratio, 1.09) selected from the gene bank of Juntendo University. Some of the selected subjects had been confirmed negative for *SNCA, PARK2, PINK1, PARK7, LRRK2*, and *PLA2G6* mutations.[9–14] From the same gene bank, we also selected 579 healthy Japanese subjects without a family history of parkinsonism (age at sampling, 58.0 ± 9.3 years; range, 23–89 years; F/M ratio, 1.54). The criteria for the diagnosis of PD were adopted by the participating neurologists and were established based on the United Kingdom Parkinson's Disease Society Brain Bank.[15]

Genetic Analysis

Genomic DNA was extracted from peripheral blood using a standard protocol. Patients with ADPD and SPD were examined for the following 7 variants: p.M57I (exon 3), p.I241M (exon 7), p.P316S (exon 9), p.R524W (exon 13), p.D620N (exon 15), p.A737V (exon 16), and p.L774M (exon 17) of *VPS35* (RefSeq accession number NM_018206.4). PCR direct sequencing was performed using a BigDye Terminator v1.1 Cycle Sequencing kit and 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) or 3730 DNA Analyzer (Applied Biosystems). In addition, SPD patients and control subjects were also genotyped for c.1858G>A (p.D620N) mutation by high-resolution melting (HRM) analysis using LightScanner and LCGreen plus (Idaho Technology, Salt Lake City, UT). HRM analysis was performed using a previously described protocol[16] and the following primers: forward, GAGGATGGTTGGTCCTTGAA; reverse, TGCCAATGATCAAGGTGATG. All exons of *VPS35* were also analyzed in patients with the p.D620N mutation using the method described previously.[3]

Haplotype analysis of the *VPS35* flanking region was performed using 3130 Genetic analyzer and GeneMapper software (Applied Biosystems, Foster City, CA). To adjust the size of PCR products, we also genotyped Centre d'Étude du Polymorphisme Humain (CEPH) control samples (1331-01 and 1331-02) for comparison of haplotypes with previously reported patients carrying the p.D620N mutation. The sequences of the PCR primers were reported previously.[3]

Results

Detection of p.D620N Mutation

We detected the heterozygous missense p.D620N mutation in 3 unrelated patients with ADPD and 1

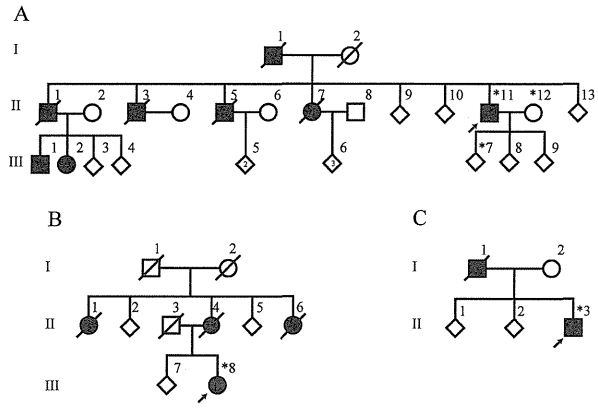

FIG. 1. Pedigrees of families with the *VPS35* p.D620N mutation (open symbol, unaffected family member; closed symbol, affected member; arrow, proband; asterisk, individual analyzed for the p.D620N mutation and/or haplotype; forward slash through symbol, deceased individuals; square, male; circle, female; diamond, unspecified sex).

patient with SPD (Fig. 1). The p.D620N has been reported previously as a pathogenic mutation for familial PD.[3,4,6] This mutation was not found in 1158 control chromosomes. Patients carrying the p.D620N mutation did not have any other variants in all exons of *VPS35*. In our population, the incidence of the p.D620N mutation was 1.0% (3 of 300) in ADPD and 0.23% (1 of 433) in SPD. The remaining variants analyzed in this study were not identified in any patients.

Haplotype analysis demonstrated that the Japanese patients with the p.D620N mutation had different genotypes from those of white patients with the same mutation.[3] One disease allele was detected by analyzing patient AII-11 and his relatives. Patients AII-11 and BIII-8 in this study carried at least the same single allele of microsatellites in the flanking region of *VPS35* (Table 1). On the other hand, patients CII-3 and D had a different genotype of D16S3105, with a locus mapped very close to *VPS35*, compared with the disease allele of AII-11 (Table 1, boldface).

TABLE 1. Haplotype analysis of VPS35 p.D620N mutation carriers

	Patient ID			
Microsatellite	AII-11	BIII-8	CII-3	D
D16S401	170	166/170	166/172	166/170
D16S3068	143	141/145	145/147	145/145
D16S753	**272**	**272**/268	268/276	264/268
VPS35 p.D620N	A	A/G	A/G	A/G
Chr16_45.333M	**294**	**294**/298	294/300	294/304
D16S3105	**191**	**191**/189	189/193	187/187
Chr16_45.615M	**147**	**147/147**	147/145	147/145
Chr16_45.806M	**246**	**246**/238	246/244	246/244
Chr16_45.835M	**237**	**237/237**	237/237	237/237
Chr16_45.855M	**212**	**212**/210	210/210	210/216
D16S3044	**195**	**195/195**	195/197	197/197

Both alleles are shown when markers of phase could not be determined.

TABLE 2. Clinical features of patients with p.D620N mutation

	Patient ID			
	AII-11	BIII-8	CII-3	D
Age at disease onset (y)	62	55	34	42
Disease duration (y)	15	2	7	21
Resting tremor	+	+	+	+
Bradykinesia	+	+	+	+
Rigidity	+	+	+	+
Gait disturbance	+	−	−	+
Postural instability	+	−	−	+
Clinical response to levodopa	+	+	+	+
Wearing off	+	−	+	+
Asymmetry at onset	+	+	+	+
Orthostatic hypotension	+	−	−	−
Incontinence	+	−	−	−
Urinary urgency	−	−	−	−
Levodopa-induced dyskinesia	+	−	+	+
Sleep benefit	+	−	+	Unknown
Dystonia at onset	−	−	−	−
Hyperreflexia	−	−	−	−
Hallucination	−	−	−	−
Other psychosis	−	−	−	−
Dementia	+	−	−	−
Gaze palsy	−	−	−	−
Brain MRI	WNL	WNL	WNL	WNL
Cardiac MIBG scintigraphy	H/M ratio (E/L), 2.38/2.68; washout ratio, 4.15%[a]	Not performed	Not performed	Not performed

[a]MIBG scintigraphy was performed when AII-11 was 76 years old.
WNL, within normal limit; H/M ratio, heart-to-mediastinum ratio; (E/L), early/late stage.

Clinical Presentation

Table 2 summarizes the clinical features of the 4 *VPS35* mutation-positive patients. Patient AII-11 was a 77-year-old man who developed right upper limb rest tremor at age 62. At age 75, he underwent gastrostomy for progressive dysphagia, then developed cognitive dysfunction without hallucination. Single-photon emission computed tomography of cerebral blood flow showed no reduction in blood flow in the basal ganglia. His father and 4 of 8 siblings were diagnosed with PD (Fig. 1A) and presented levodopa-responsive typical parkinsonism: upper limb tremor and small-step gait. His nephew and niece were also diagnosed with PD, and the nephew developed parkinsonism in his early fifties. Patients BIII-8 and CII-3 both developed upper limb rest tremor at ages 34 and 55, respectively. The mother and aunts of patient BIII-8

and the father of patient CII-3 also developed PD (Fig. 1B, C). Patient D, who developed upper limb rest tremor at age 42, had no family history of PD. She underwent subthalamic nucleus deep brain stimulation (STN-DBS) at age 60 because of disabling motor fluctuation and dyskinesia refractory to pharmacological treatment. All affected patients were born to nonconsanguineous parents.

Discussion

VPS35 has been reported as the pathogenic gene for ADPD, and only 1 mutation, p.D620N, has been reported in several unrelated white families. To our knowledge, there have been no reports of Asian PD patients with *VPS35* mutations.[3,8] Based on this background, we set out in this study to determine the incidence of *VPS35* mutations in Japanese patients with PD. We detected the heterozygous p.D620N mutation of *VPS35* in 3 ADPD families and 1 SPD patient with East Asian ancestry. On the other hand, we could not conclude the pathogenicity of 6 other variants that had been reported as potentially pathogenic for PD because none of the variants was detected in our patients with PD.

The frequency of the p.D620N mutation in Japanese patients was 1.0% in ADPD and 0.23% in SPD. Although the exact frequency among whites is undetermined, the frequency is relatively higher in Japanese patients compared with that reported in previous studies (0%–1.22%).[3,4,6,7,17] Moreover, the frequency in Japanese patients also differs greatly from those of other Asian populations such as Taiwanese patients and mainland Chinese patients (0%).[3,8] Although the mutation frequency was expected to be lower than that of other pathogenic genes for ADPD, such as multiplication of *SNCA*[9,18] and point mutation of *LRRK2*,[19–21] *VPS35* may be one of the most important genes in Japanese PD. Because we screened for only 7 reported variants, we cannot determine the exact frequency of *VPS35* mutations in ADPD; we would need to analyze all 17 exons of *VPS35* in ADPD patients to screen for other variants and to assess the incidence of all disease-associated *VPS35* mutations.[3,4] Furthermore, we would need to perform mutational analysis for SPD patients, in addition to ADPD, to identify Asian population-specific variants, such as *LRRK2* p.G2385R, associated with susceptibility for PD.[19]

Based on haplotype analysis reported in previous studies, the substitution of *VPS35* c.1858G>A (p.D620N) occurs from independent mutational events.[3] We were able to determine the chromosomal phase only in patient AII-11 (family A). The p.D620N mutation possibly shared a common founder between Japanese ADPD families A and B; however, it was inconclusive because the phase of patient BIII-8 was undetermined. On the other hand, the same p.D620N mutation probably occurred independently in patient CII-3 (family C) and patient D. By genotyping of D16S3105, which is located approximately 1.5 kb centromeric of *VPS35*, there were at least 3 different haplotypes in Japanese because families A and C and patient D (SPD) did not have the same alleles for this microsatellite. To determine the chromosomal phase of families B and C, detailed genetic analyses of other family members are needed in future studies. These results suggest the existence of 3 or more founders in Japanese patients, in addition to the reported white patients with the p.D620N mutation or de novo mutations, indicating that the p.D620N mutation site is a mutational hot spot in *VPS35* across different ethnic populations.

According to previous reports, the average AAO of patients with the *VPS35* mutation was 50–60 years (50.6 ± 7.3 years),[3] with a distinctive feature of a slightly younger AAO compared with patients with idiopathic PD. In our study, the AAO was nonspecific with a wide range between 30–70 years. Because the family history of patient D was unknown, she was categorized as SPD. With regard to *VPS35* mutation penetrance, it is incomplete from the results of a previous report.[3] Therefore, although the frequency is low, patients with p.D620N mutation could be found among SPD patients.

The clinical symptoms of our patients with *VPS35* mutation closely resembled the idiopathic PD form, with tremor-dominant dopa-responsive parkinsonism. Psychiatric problems were inconspicuous; however, dementia may occur in patients with a long disease course, similar to patient AII-11, who had PD for 15 years. Our patients with *VPS35* mutations had normal brain MRI and cardiac MIBG scintigraphy. There have been no definite pathological mutations of *VPS35* in the spectrum of LB disorders. On the basis of these results, patients with *VPS35* mutation could show comparatively benign disease course without widespread LBs pathology.[22,23]

VPS35 assembles into the retromer cargo-recognition complex that associates with the cytosolic face of the endosomes. The retromer mediates the retrograde transport of transmembrane cargo from the endosomes to the trans-Golgi network.[5] The p.D620N mutation of *VPS35* might cause impairment of interaction with other components of the retromer complex and impaired retrograde trafficking of recycling proteins,[4] similar to α-synuclein and LRRK2, which are involved in vesicle trafficking.[24,25] Mutations in familial PD genes, including *VPS35*, may cause disruption of intracellular trafficking and lead to neurodegeneration. These findings suggest that impairment of intracellular trafficking systems is associated with the pathogenesis of PD. Although the association between the p.D620N mutation of *VPS35* and PD remains unknown, further functional studies might shed light on the pathogenesis

of *VPS35* mutation and the effects of interaction with other known pathogenic gene products on PD.

In conclusion, we have reported Asian PD patients with the *VPS35* p.D620N mutation. The p.D620N substitution may be a mutational hot spot across different ethnic populations. The frequency of *VPS35* mutation was low in ADPD; however, it is relatively high in Japanese patients compared with that reported in other populations.[3,4,6–8] Based on the clinical features of patients with *VPS35* mutation, *VPS35* should be analyzed in patients with PD, especially ADPD or tremor-predominant PD. ■

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References

1. Lang AE, Lozano AM. Parkinson's disease. First of two parts. N Engl J Med 1998;339:1044–1053.
2. Lesage S, Brice A. Parkinson's disease: from monogenic forms to genetic susceptibility factors. Hum Mol Genet 2009;18: R48–R59.
3. Vilariño-Güell C, Wider C, Ross OA, et al. VPS35 mutations in Parkinson disease. Am J Hum Genet 2011;89:162–167.
4. Zimprich A, Benet-Pagès A, Struhal W, et al. A mutation in VPS35, encoding a subunit of the retromer complex, causes late-onset Parkinson disease. Am J Hum Genet 2011;89:168–175.
5. Bonifacino JS, Hurley JH. Retromer. Retromer. Curr Opin Cell Biol 2008;4:427–436.
6. Sheerin UM, Charlesworth G, Bras J, et al. Screening for VPS35 mutations in Parkinson's disease. Neurobiol Aging 2012;4: 838.e1–e5.
7. Guella I, Soldà G, Cilia R, et al. The Asp620asn mutation in VPS35 is not a common cause of familial Parkinson's disease. Mov Disord 2012;27:800–801.
8. Zhang Y, Chen S, Xiao Q, et al. Vacuolar protein sorting 35 Asp620Asn mutation is rare in the ethnic Chinese population with Parkinson's disease. Parkinsonism Relat Disord 2012;18:638–640.
9. Nishioka K, Hayashi S, Farrer MJ, et al. Clinical heterogeneity of alpha-synuclein gene duplication in Parkinson's disease. Ann Neurol 2006;59:298–309.
10. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605–608.
11. Kumazawa R, Tomiyama H, Li Y, et al. Mutation analysis of the PINK1 gene in 391 patients with Parkinson disease. Arch Neurol 2008;65:802–808.
12. Tomiyama H, Li Y, Yoshino H, et al. Mutation analysis for DJ-1 in sporadic and familial parkinsonism: screening strategy in parkinsonism. Neurosci Lett 2009;455:159–161.
13. Tomiyama H, Li Y, Funayama M, et al. Clinicogenetic study of mutations in LRRK2 exon 41 in Parkinson's disease patients from 18 countries. Mov Disord 2006;21:1102–1108.
14. Yoshino H, Tomiyama H, Tachibana N, et al. Phenotypic spectrum of patients with PLA2G6 mutation and PARK14-linked parkinsonism. Neurology 2010;75:1356–1361.
15. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181–184.
16. Funayama M, Tomiyama H, Wu RM, et al. Rapid screening of ATP13A2 variant with high-resolution melting analysis. Mov Disord 2010;25:2434–2437.
17. Lesage S, Condroyer C, Klebe S et al. Identification of VPS35 mutations replicated in French families with Parkinson disease. Neurology 2012;78:1449–1450.
18. Ibáñez P, Lesage S, Janin S, et al. Alpha-synuclein gene rearrangements in dominantly inherited parkinsonism: frequency, phenotype, and mechanisms. Arch Neurol 2009;66:102–108.
19. Seki N, Takahashi Y, Tomiyama H, et al. Comprehensive mutational analysis of LRRK2 reveals variants supporting association with autosomal dominant Parkinson's disease. J Hum Genet 2011;56:671–675.
20. Di Fonzo A, Rohe C. F, Ferreira J, et al. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. Lancet 2005;365:412–415.
21. Gilks WP, Abou-Sleiman PM, Gandhi S, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005;365:415–416.
22. Orimo S, Amino T, Yokochi M, et al. Preserved cardiac sympathetic nerve accounts for normal cardiac uptake of MIBG in PARK2. Mov Disord 2005;10:1350–1353.
23. Verstraeten A, Wauters E, Crosiers D, et al. Contribution of VPS35 genetic variability to LBD in the Flanders-Belgian population. Neurobiol Aging 2012;33:e11–e13.
24. Caudle WM, Colebrooke RE, Emson PC, et al. Altered vesicular dopamine storage in Parkinson's disease: a premature demise. Trends Neurosci 2008;31:303–308.
25. Berwick DC, Harvey K. LRRK2 signaling pathways: the key to unlocking neurodegeneration? Trends Cell Biol 2011;21:257–265.

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Brief communication

Analysis of *C9orf72* repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis

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Abstract

Recently, a hexanucleotide repeat expansion in *C9orf72* was identified as the most common cause of both sporadic and familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia in Western populations. We analyzed 563 Japanese patients with ALS (552 sporadic and 11 familial) using fluorescent fragment-length analysis of *C9orf72* and repeat-primed polymerase chain reaction analysis. Haplotype analysis was performed for 42 single nucleotide polymorphisms in patients with *C9orf72* repeat expansion. *C9orf72* repeat expansion was found in 2 patients with sporadic ALS (2/552 = 0.4%) and no patients with familial ALS (0/11 = 0%). In the probands' families, 1 primary progressive aphasia patient and 1 asymptomatic 76-year-old individual exhibited *C9orf72* repeat expansion. All of the patients with the *C9orf72* repeat expansion carried the 20-single nucleotide polymorphism consensus risk haplotype. The frequency of the *C9orf72* repeat expansion among Japanese patients is much lower than in Western populations. The existence of a 76-year-old asymptomatic carrier supported the notion of incomplete penetrance. The *C9orf72* mutation should be analyzed in sporadic ALS patients after determining their family histories not only of frontotemporal dementia but also of primary progressive aphasia.

Keywords: Amyotrophic lateral sclerosis; *C9orf72*; Incomplete penetrance; Sporadic; Aphasia; Frontotemporal dementia

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that primarily affects motor neurons in the spinal cord, brain stem, and cerebral cortex, typically leading to death within a few years. Five to ten percent of ALS cases are familial, and the remaining cases are believed to be sporadic (Valdmanis et al., 2009). A number of genes causing ALS with a dominant mode of inheritance have

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been discovered, such as *SOD1*, *TARDBP*, *FUS*, *VAPB*, *ANG*, *VCP*, *OPTN* (Ticozzi et al., 2011), and *UBQLN2* (Deng et al., 2011). Moreover, there is increasing clinical and pathological evidence for the hypothesis that ALS and frontotemporal dementia (FTD) constitute an overlapping continuum of diseases (Lomen-Hoerth et al., 2002; Neumann et al., 2006). Recently, the expansion of a noncoding GGGGCC hexanucleotide repeat in the *C9orf72* gene has been reported to be a major cause of both ALS and FTD (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011) and the most common genetic abnormality in familial and sporadic forms of both ALS and FTD, particularly in Western populations (Chiò et al., 2012; DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011; Sabatelli et al., 2012; Stewart et al., 2012). In the present study, we describe the incidence and demographic and clinical features associated with the *C9orf72* mutation in a large cohort of Japanese ALS patients. We also perform haplotype analysis to investigate whether Japanese patients have the same risk haplotype as European patients (Gijselinck et al., 2012; Laaksovirta et al., 2010; Mok et al., 2012).

2. Methods

2.1. Subjects

We obtained a total of 760 DNA samples from the Japanese Consortium for Amyotrophic Lateral Sclerosis Research (JaCALS; Appendix A). A total of 563 (11 familial and 552 sporadic) patients were diagnosed with ALS according to the El Escorial revised criteria (Brooks et al., 2000) and classified as bulbar-onset, spinal-onset, FTD-ALS, or other (see Supplementary Table 1 for details). We had determined the family histories of ALS but not FTD or primary progressive aphasia (PPA) in all of the patients when they were enrolled as patients with sporadic ALS (SALS). We recruited 197 control subjects, none of whom had a medical or family history of neurodegenerative disorders. The mean age at onset of the patients with ALS was 60.4 ± 11.7 years (range 20–86), and the mean age at sampling of the controls was 60.6 ± 10.3 years (range 26–83). All of the subjects were unrelated Japanese individuals. Written informed consent was obtained from all of the subjects. The ethical committees at the participating institutions approved this study.

2.2. Fluorescent fragment-length analysis of C9orf72 and repeat-primed PCR analysis

The normal repeat number of the GGGGCC hexanucleotide was determined in all of the patients and control subjects using genotyping primers, as previously described (DeJesus-Hernandez et al., 2011). To provide a qualitative assessment of the presence of *C9orf72* repeat expansions, we performed repeat-primed polymerase chain reaction (PCR), as previously described (DeJesus-Hernandez et al., 2011).

2.3. Haplotype analysis

We genotyped 42 single nucleotide polymorphisms (SNPs) across 232 kilobase of Chromosome 9p21, which were first described as the founder haplotype in the Finnish ALS population (Laaksovirta et al., 2010), using primers (Supplementary Table 2) to determine whether our Japanese patients carried the haplotype associated with a risk of ALS. These 42 SNPs included the 20-SNP consensus risk allele that had recently been detected in genome-wide association studies in several populations (Mok et al., 2012). We also performed haplotype analysis with 4 microsatellites (D9S1121, D9S169, D9S270, and D9S104) flanking the *C9orf72* GGGGCC repeat, as previously described (Gijselinck et al., 2012) (Fig. 1).

3. Results

3.1. Detection of C9orf72 repeat expansion

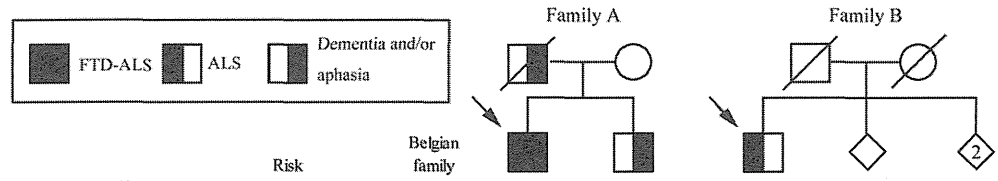
The *C9orf72* repeat expansion was found in 2 of 522 Japanese patients (2/552 = 0.4%) with SALS and none of the 11 patients (0/11 = 0%) with familial ALS (FALS) using repeat-primed PCR (Table 1). Patient A-I with a *C9orf72* mutation was classified as SALS in this study, but after detecting the mutation, we found that patient A-II (a brother of patient A-I) developed aphasia and dementia and had a *C9orf72* mutation (Fig. 1). The average repeat number based on fluorescent fragment-length analysis was 3.65 ± 2.43 (range 2–13 repeats) in 561 ALS patients without the *C9orf72* mutation. A subsequent analysis of 197 healthy controls did not detect any *C9orf72* mutation. The average repeat number was 3.69 ± 2.46 (range 2–14 repeats) in the 197 controls. The mean age at disease onset in patients with *C9orf72* mutation, including patient A-II, was 64.7 ± 6.1 years (range 57–72). The genotypes of all individuals with the *C9orf72* mutation were detected for the 20 SNPs spanning a 140-kilobase segment concordant with the recently identified risk haplotype on chromosome 9p (Mok et al., 2012) and 24 or 25 consecutive SNPs in the 42-SNP Finish risk haplotype (Laaksovirta et al., 2010) (Fig. 1, Supplementary Table 3).

3.2. Clinical presentations of individuals with C9orf72 mutation

3.2.1. Patient A-I (family A)

Patient A-I was a 65-year-old man who reported weakness in the left leg. The weakness progressed, and he developed fasciculation. At age 66, a neurological examination revealed dementia. His Mini Mental State Examination score was 23/30, and his Frontal Assessment Battery score was 13/18. He also exhibited dysarthria and weakness, atrophy, and fasciculation in the tongue and all 4 modalities. His tendon reflexes were diminished, and the plantar re-

A)



B)

Marker	Position	Risk allele	Freq JPT	Belgian family DR14	Patient A-I	Patient A-II	Patient B-I	Subject B-II
rs725804	27458939	A	A=0.081		C/C	C/C	C/C	C/C
rs10511816	27468461	T	T=0.105		T/G	T/G	G/G	G/G
rs1444533	27477874	A	A=0.553		A/A	A/A	A/G	A/G
rs1822723*	27478052	C	C=0.669		C/C	C/C	C/C	C/C
rs4879515*	27482235	T	T=0.645		T/T	T/T	T/T	T/T
rs895023	27483959	T	T=1.000		T/T	T/T	T/T	T/T
rs868856*	27489251	T	T=0.247		T/T	T/T	T/T	T/T
rs7046653*	27490967	A	A=0.250		A/A	A/A	A/A	A/A
rs2440622	27495418	A	A=0.988		A/A	A/A	A/A	A/A
rs1977661 *	27502986	C	C=0.651		C/C	C/C	C/C	C/C
rs903603*	27529316	C	C=0.341		C/C	C/C	C/T	C/T
rs10812610*	27533984	C	A=0.680		C/C	C/C	C/A	C/A
rs2814707*	27536397	A	A=0.058		A/A	A/A	A/G	A/G
rs3849942*	27543281	A	A=0.076		A/A	A/A	A/G	A/G
C9orf72	27546544..27573842			mutation	mutation	mutation	mutation	mutation
rs12349820	27553876	T	T=0.890		T/T	T/T	T/T	T/T
rs10122902*	27556780	G	G=0.738		G/G	G/G	G/G	G/G
rs10757665*	27557919	T	T=0.890		T/T	T/T	T/T	T/T
rs1565948*	27559733	G	G=0.600		G/G	G/G	G/G	G/G
rs774359*	27561049	C	C=0.070		C/C	C/C	C/T	C/T
rs2282241*	27572255	G	G=0.326		G/G	G/G	G/T	G/T
rs1948522*	27575785	C	C=0.837		C/C	C/C	C/C	C/C
rs1982915*	27579560	G	G=0.238		G/G	G/G	G/A	G/A
rs2453556*	27586162	G	G=0.448		G/G	G/G	G/A	G/A
rs702231*	27588731	A	A=0.634		A/A	A/A	A/A	A/A
rs696826*	27589657	G	G=0.983		G/G	G/G	G/G	G/G
rs2477518*	27599746	T	T=0.820		T/T	T/T	T/T	T/T
D9S270	28404862 .. 28404948			327	327/325	327/325	323/323	323/323
D9S104	28771379 .. 28771576			168	158/158	158/158	158/171	158/171

Fig. 1. (A) The pedigrees of the 2 families with *C9orf72* repeat expansion. To maintain confidentiality, several unaffected individuals who died early in families A and B are not shown. Probands are indicated by arrows. (B) The genotyping data of the single nucleotide polymorphisms (SNPs) and microsatellites. Twenty SNPs, which comprised a recently identified consensus risk haplotype (Mok et al., 2012), are shown with an asterisk. See Supplementary Table 3 for details of the analyses of 42 SNPs (Laaksovirta et al., 2010) and microsatellites (Gijselinck et al., 2012). Alleles possibly shared between our subjects and patients in Western populations are shown in bold. The genotypes of all 4 subjects with respect to the 20 SNPs were found to be concordant with the risk haplotype (Mok et al., 2012). All of the positions of SNPs and microsatellites were from NC_000009.11. Abbreviations: ALS, amyotrophic lateral sclerosis; Freq JPT, Frequency in Japanese in Tokyo from International HapMap project (International HapMap Consortium, 2003); FTD, frontotemporal dementia.

sponse was extensor on the left. He had neither dysphagia nor dyspnea. No sensory abnormalities were noted. Extensive screening for causes of motor neuropathy was negative. The diagnosis was clinically probable ALS-laboratory supported (Brooks et al., 2000) and FTD-ALS.

3.2.2. *Patient A-II (family A)*

This patient was a 57-year-old man who presented with difficulty speaking. He was believed to have suffered from a mental disease after being imprisoned because of his involvement in a fatal car accident. At age 64, he was severely dysfluent and could barely speak. Logoclonia was particularly prominent. However, he did not exhibit any violent behavior or other behavioral abnormalities. He also did not display any clinical features of motor neuron disease. Brain magnetic resonance imaging revealed severe frontotemporal lobar atrophy. PPA was considered the most likely diagnosis.

3.2.3. *Patient B-I (family B)*

Patient B-I was a 72-year-old man who presented with gait disturbance and weakness in the proximal lower extremity muscle. His family history was negative for motor neuron disease and dementia (Fig. 1). The muscle weakness and atrophy progressed and spread to the other parts of his body despite treatment with intravenous gamma globulin. At age 74, he could not roll over while sleeping. A neurological examination showed marked muscle atrophy in his arms and shoulders and prominent fasciculation in his legs. The deep tendon reflexes were decreased in his limbs, and he had no pathological reflexes. Sensations in all 4 modalities were intact. At age 75, he developed dyspnea and dysphagia and started noninvasive positive pressure ventilation and intravenous hyperalimentation. He died of respiratory insufficiency at age 76. An autopsy was not performed. The diagnosis was clinically suspected ALS (Brooks et al., 2000).