ИНФОРМАЦИЯ ЗА:

Наименование на заболяването

Прогресивна супрануклеарна парализа

Определение на заболяването

Прогресивната супрануклеарна парализа е рядко невродегенеравно заболяване с късно начало. Заболяването се характеризира с развитието на супрануклеарна погледна пареза, постурална нестабилност, прогресираща ригидност и лека деменция.

Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)

G23.1

Код на заболяването по Orpha code

ORPHA683

Епидемиологични данни за заболяването в Република България

Предполага се честота 1-9 / 100 000, както в останалите страни от Европа.

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

1. Nath, U., Ben-Shlomo, Y., Thomson, R. G., Lees, A. J., Burn, D. J. Clinical features and natural history of progressive supranuclear palsy: a clinical cohort study. Neurology 60: 910-916, 2003.
2. Donker Kaat, L., Boon, A. J. W., Azmani, A., Kamphorst, W., Breteler, M. M. B., Anar, B., Heutink, P., van Swieten, J. C. Familial aggregation of parkinsonism in progressive supranuclear palsy. Neurology 73: 98-105, 2009.

Епидемиологични данни за заболяването в Европейския съюз

1-9 / 100 000; Предполага се болестност около 1/16,600.

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

1. Nath, U., Ben-Shlomo, Y., Thomson, R. G., Lees, A. J., Burn, D. J. Clinical features and natural history of progressive supranuclear palsy: a clinical cohort study. Neurology 60: 910-916, 2003.
2. Donker Kaat, L., Boon, A. J. W., Azmani, A., Kamphorst, W., Breteler, M. M. B., Anar, B., Heutink, P., van Swieten, J. C. Familial aggregation of parkinsonism in progressive supranuclear palsy. Neurology 73: 98-105, 2009.

Оценка на съответствието на заболяването с дефиницията за рядко заболяване съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето

Заболяването е с разпространение под 5/ 10 000 души от населението на Европейския съюз.

Критерии за диагностициране на заболяването

Диагностициране на заболяването (дефиниция на случай):

Признаците и симптомите на заболяването: Прогресивната супрануклеарна пареза

(ПСП) е с начало обикновено около шесто или седмо десетилетие. Описани са пет клинични варианта с клиникопатологични корелати: Класически ПСП (Richardson синдром) и четири атипични варианта на ПСП, включващи: ПСП- Паркинсонизъм (PSP-P), ПСП- чиста акинезия с фрийзинг феномени (PSP-PAGF), ПСП-кортикобазален синдром (PSP-CBS) и ПСП-прогресивна нефлуентна афазия (PSP-PNFA). Richardson синдромът е най-честият клиничен вариант и се манифестира с нестабилна походка, падания, дължащи се на постуралната нестабилност, когнитивни нарушения и забавеност на вертикалните очни сакади. Прогресивно пациентите развиват други проблеми, като говорни нарушения, вертикална погледна пареза и трудности при преглъщане.

ПСП-паркинсонизъм (PSP-P) се характеризира по-скоро с ранно изразен паркинсонизъм (тремор, брадикинезия в крайниците, ригидност - аксиална и в крайниците) отколкото с падания и когнитивни промени. В хода на годините пациентите накрая развиват клинични черти, характерни за Richardson синдром.

ПСП-чиста акинезия с фрийзинг феномени (PSP-PAGF) се характеризира с прогресиращи фрийзинг феномени, засягане на говора и писането рано в хода на заболяването. По-късно развитие на аксиална ригидност, лицева хипомимия и впоследствие евентуално супрануклеарна погледна пареза надолу.

ПСП-кортикобазален синдром (PSP-CBS) се характеризира с прогресивна, асиметрична диспраксия, ригидност на крайниците, брадикинезия и прогресивна постурална нестабилност.

ПСП- прогресивна нефлуентна афазия (PSP-PNFA) се характеризира с нарушения в говора (говорна апраксия, аграматизъм, фонемни грешки). Моторни симптоми се появяват по-късно в хода на заболяването.

Nicholl и колеги (2003) описват и форма на ПСП, характеризираща се с фатална дихателна хиповентилация при двама пациенти с кръвнородствена връзка на родителите. При двамата пациенти се установява H1/H1 хаплотип, които се асоциира с ПСП.

Етиологията и патогенезата: ПСП е 4R таупатия, съставена от преобладаване на четири повтора (екзон 10 полозитивни) тау изоформи и характерен биохимичен профил (дублет тау 64 и тау 69). MAPT H1c специфичен хаплотип е рисков фактор за развитие на заболяването. ПСП се характеризира също с дефицити в няколко невротрансмитерни системи (в това число допаминергична, холинергична, габаергична). Фактори, които отключват тау-невродегенерацията са неизвестни.

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

1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
2. Mata, M., Dorovini-Zis, K., Wilson, M., Young, A. B. New form of familial Parkinson-dementia syndrome: clinical and pathologic findings. Neurology 33: 1439-1443, 1983.
3. Ohara, S., Kondo, K., Morita, H., Maruyama, K., Ikeda, S., Yanagisawa, N. Progressive supranuclear palsy-like syndrome in two siblings of a consanguineous marriage. Neurology 42: 1009-1014, 1994.
4. Pastor, P., Pastor, E., Carnero, C., Vela, R., Garcia, T., Amer, G., Tolosa, E., Oliva, R. Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene. Ann. Neurol. 49: 263-267, 2001.

5. Nath, U., Ben-Shlomo, Y., Thomson, R. G., Lees, A. J., Burn, D. J. Clinical features and natural history of progressive supranuclear palsy: a clinical cohort study. Neurology 60: 910-916, 2003.
6. Donker Kaat, L., Boon, A. J. W., Azmani, A., Kamphorst, W., Breteler, M. M. B., Anar, B., Heutink, P., van Swieten, J. C. Familial aggregation of parkinsonism in progressive supranuclear palsy. Neurology 73: 98-105, 2009.
7. Nicholl, D. J., Greenstone, M. A., Clarke, C. E., Rizzu, P., Crooks, D., Crowe, A., Trojanowski, J. Q., Lee, V. M.-Y., Heutink, P. An English kindred with a novel recessive tauopathy and respiratory failure. Ann. Neurol. 54: 682-686, 2003.
8. Quadros, A., Weeks, O. I., Ait-Ghezala, G. Role of tau in Alzheimer's dementia and other neurodegenerative diseases. J. Appl. Biomed. 5: 1-12, 2007.
9. Baker, M., Litvan, I., Houlden, H., Adamson, J., Dickson, D., Perez-Tur, J., Hardy, J., Lynch, T., Bigio, E., Hutton, M. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. Hum. Molec. Genet. 8: 711-715, 1999.
10. Litvan, I., Baker, M., Hutton, M. Tau genotype: no effect on onset, symptom severity, or survival in progressive supranuclear palsy. Neurology 57: 138-140, 2001.

Алгоритми за диагностициране на заболяването

Алгоритми за диагностициране на заболяването: Съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Анамнезата: Прогресивната супрануклеарна пареза (ПСП) е с начало обикновено около шесто или седмо десетилетие. Описани са пет клинични варианта с клиникопатологични корелати: Класически ПСП (Richardson синдром) и четири атипични варианта на ПСП, включващи: ПСП- Паркинсонизъм (PSP-P), ПСП- чиста акинезия с фрийзинг феномени (PSP-PAGF), ПСП-кортикобазален синдром (PSP-CBS) и ПСП-прогресивна нефлуентна афазия (PSP-PNFA). Richardson синдромът е най-честият клиничен вариант и се манифестира с нестабилна походка, падания, дължащи се на постуралната нестабилност, когнитивни нарушения и забавеност на вертикалните очни сакади. Прогресивно пациентите развиват други проблеми, като говорни нарушения, вертикална погледна пареза и трудности при преглъщане.

ПСП-паркинсонизъм (PSP-P) се характеризира по-скоро с изразен ранно паркинсонизъм (тремор, брадикинезия в крайниците, ригидност - аксиална и в крайниците) отколкото с падания и когнитивни промени. В хода на годините, пациентите накрая развиват клинични черти характерни за Richardson синдром.

ПСП-чиста акинезия с фрийзинг феномени (PSP-PAGF) се характеризира с прогресиращи фрийзинг феномени, засягане на говора и писането рано в хода на заболяването. По-късно развитие на аксиална ригидност, лицева хипомимия и в последствие евентуално супрануклеарна погледна пареза надолу.

ПСП-кортикобазален синдром (PSP-CBS) се характеризира с прогресивна, асиметрична диспраксия, ригидност на крайниците, брадикинезия и прогресивна постурална нестабилност.

ПСП- прогресивна нефлуентна афазия (PSP-PNFA) се характеризира с нарушения в говора (говорна апраксия, аграматизъм, фонемни грешки). Моторни симптоми се появяват по-късно в хода на заболяването.

Диференциалната диагноза на заболяването: Паркинсонова болест; други атипични паркинсонови заболявания, като мултиситемна атрофия и кортикобазалната дегенерация; Niemann-Pick заболяването тип C; Whipple заболяването.

Лабораторни, образни и хистологични изследвания: Piccini и колеги (2001) показват при PET изследване значително намаление на натрупването в кауда и путамен на (18)F-dopa, наред със значима редукция глюкозния метаболизъм в стриатум, латерална и медиална препоторна кора и дорзална префронтална кора. ПСП се характеризира невропатологично с невронална загуба, глиоза с астроцитни плаки и акумулиране на тау-имунореактивни неврофибрилерни натрупвания в специфични мозъчни региони. Разликите в степента и областите на натрупване на фосфорилирания тау протеин корелират с петте клинични варианти на заболяването.

Генетични изследвания и медико-генетично консултиране: De Yebenes и колеги (1995) описват 7 семейства с множество засегнати индивиди, като при 3 фамилии се предполага автозомно-доминантно унаследяване и при 1 семейство с кръвнородствена връзка на родителите автозомно-рецесивно унаследяване. Въпреки че множеството от случаите с ПСП са спорадични, е възможно да има редки генетично определени форми. Форма на атипична погледна пареза се причинява от мутация в микротубуло-асоциирания протеин тау ген (MAPT) на хромозома 17q21.31, който се унаследява на АР. Авторите предполагат непълна пенетрантност на тази форма на заболяването поради факта, че са установени хетерозиготни носители, които не развиват заболяването или развиват класическа с късно начало Допа-отговаряща Паркинсонова болест. Прогресивната супрануклеарна парализа-1 (PSNP1) би могла да се дължи на хетерозиготна мутация на гена, кодиращ микротубулно-асоцииран тау протеин (MAPT) на хромозома 17 по АД тип на унаследяване. Други локуси за ПСП са картирани в хромозома 1q31 (PSNP2) и 11p12-P11 (PSNP3).

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1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
2. Mata, M., Dorovini-Zis, K., Wilson, M., Young, A. B. New form of familial Parkinson-dementia syndrome: clinical and pathologic findings. Neurology 33: 1439-1443, 1983.
3. Ohara, S., Kondo, K., Morita, H., Maruyama, K., Ikeda, S., Yanagisawa, N. Progressive supranuclear palsy-like syndrome in two siblings of a consanguineous marriage. Neurology 42: 1009-1014, 1994.
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5. Rossi, G., Gasparoli, E., Pasquali, C., Di Fede, G., Testa, D., Albanese, A., Bracco, F., Tagliavini, F. Progressive supranuclear palsy and Parkinson's disease in a family with a new mutation in the tau gene. (Letter) Ann. Neurol. 55: 448 only, 2004.
6. Rojo, A., Pernaute, R. S., Fontan, A., Ruiz, P. G., Honnorat, J., Lynch, T., Chin, S., Gonzalo, I., Rabano, A., Martinez, A., Daniel, S., Pramstaller, P., Morris, H., Wood, N., Lees, A., Tabernero, C., Nyggard, T., Jackson, A. C., Hanson, A., de Yebenes, J. G. Clinical genetics of familial progressive supranuclear palsy. Brain 122: 1233-1245, 1999.
7. Ros, R., Thobois, S., Streichenberger, N., Kopp, N., Sanchez, M. P., Perez, M., Hoenicka, J., Avila, J., Honnorat, J., de Yebenes, J. G. A new mutation of the tau gene, G303V, in early-onset familial progressive supranuclear palsy. Arch. Neurol. 62: 1444-1450, 2005.

8. Nath, U., Ben-Shlomo, Y., Thomson, R. G., Lees, A. J., Burn, D. J. Clinical features and natural history of progressive supranuclear palsy: a clinical cohort study. Neurology 60: 910-916, 2003.
9. Donker Kaat, L., Boon, A. J. W., Azmani, A., Kamphorst, W., Breteler, M. M. B., Anar, B., Heutink, P., van Swieten, J. C. Familial aggregation of parkinsonism in progressive supranuclear palsy. Neurology 73: 98-105, 2009.
10. Piccini, P., de Yebenez, J., Lees, A. J., Ceravolo, R., Turjanski, N., Pramstaller, P., Brooks, D. J. Familial progressive supranuclear palsy: detection of subclinical cases using (18)F-dopa and (18)fluorodeoxyglucose positron emission tomography. Arch. Neurol. 58: 1846-1851, 2001.
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12. Rojo, A., Pernaute, R. S., Fontan, A., Ruiz, P. G., Honnorat, J., Lynch, T., Chin, S., Gonzalo, I., Rabano, A., Martinez, A., Daniel, S., Pramstaller, P., Morris, H., Wood, N., Lees, A., Tabernero, C., Nyggard, T., Jackson, A. C., Hanson, A., de Yebenes, J. G. Clinical genetics of familial progressive supranuclear palsy. Brain 122: 1233-1245, 1999.
13. Donker Kaat, L., Boon, A. J. W., Azmani, A., Kamphorst, W., Breteler, M. M. B., Anar, B., Heutink, P., van Swieten, J. C. Familial aggregation of parkinsonism in progressive supranuclear palsy. Neurology 73: 98-105, 2009.
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Алгоритми за лечение на заболяването

Алгоритми за лечение на заболяването: Съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Терапевтичните подходи към заболяването, в това число консервативни и оперативни, техните предимства, рискове и очаквана ефективност: Все още няма терапия, която да излекува дефинитивно заболяването. Някои лекарства, в зависимост от клиничния вариант, редуцират болестността и подобряват качеството на живот (например леводопа отговора на пациенти с ПСП-паркинсонизъм). Amantadine може да подобри фрийзинг феномените и други антихолинергични лекарства понякога подобряват глас и нарушения на речта.

Препоръчителен диетичен режим и физическа активност и др.:

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

1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
2. Nath, U., Ben-Shlomo, Y., Thomson, R. G., Lees, A. J., Burn, D. J. Clinical features and natural history of progressive supranuclear palsy: a clinical cohort study. Neurology 60: 910-916, 2003.

Алгоритми за проследяване на заболяването
Алгоритми за проследяване на заболяването (Необходимостта от последващи болнични и извънболнични грижи; Необходимостта от консултации с други специалисти): Съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.   Прогнозата на заболяването: Поради чести падания пациентите постепенно стават зависими от инвалидна количка. Затрудненията в дишането и гълтането, както и инфекциите са основните причини за смърт, обикновено 6-12 години след появата на болестта.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
Алгоритми за рехабилитация на заболяването
Алгоритми за рехабилитация на заболяването: Съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
Необходими дейности за профилактика на заболяването (ако такива са приложими)
Дейности за профилактика на заболяването: Съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба
Създаването на Национален експертен център „Редки невродегенеративни заболявания, протичащи с когнитивни, поведенчески и моторни нарушения" за диагностика, лечение и проследяване и рехабилитация включително и на пациенти с това заболявания под ръководството на чл.кор.проф.д-р Л. Трайков, дмн (национален експерт с най-голям опит и принос за диагностиката и лечението на тези заболявания).
Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)
Опитът на кандидатстващия експертен център за диагноза и лечение на редки заболявания с атипичен паркинсонизъм, като Прогресивна супрануклеарна парализа, датира от 2001 година със създаването на център за диагноза и лечение на невродегенеративни заболявания, протичащи с деменция и допълнително на център за диагноза и лечение на Паркинсонова болест. От дълги години този център е рефериран център за заболявания, протичащи с атипичен паркинсонизъм, като Прогресивна супрануклеарна парализа, особено за комплексни, редки и наследствени случаи. През годините вследствие на натрупания опит и труд, както и значителен брой

на пациенти с тези редки заболявания, реферирани към нашите два центъра, са осъществени няколко дисертации в областта: 1. Клинико-генетични корелации при невродегенеративни заболявания, протичащи с паркинсонизъм (защитена дисертация за доктор по медицина от д-р Радка Павлова, 2013 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Проучване на невропсихологичния профил при пациенти с Паркинсон плюс синдроми (защитена дисертация за доктор по медицина от д-р Силвия Скелина, 2016 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. Клинико-генетични проучвания при фронтотемпорална деменция и сродни заболявания (защитена дисертация за доктор по медицински науки от д-р Шима Мехрабиан, 2016 г.). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, с атипичен паркинсонизъм, като Прогресивна супрануклеарна парализа с подробно фенотипизиране на всеки един случай, което дава възможност за добър мониторинг на пациентите, както и изследователски анализ върху характеристиката на отделните заболявания. Дейността на центъра по отношение на диагноза и лечение на редки заболявания с атипичен паркинсонизъм, като Прогресивна супрануклеарна парализа, обхваща всички диагностични дейности съобразно новите диагностични критерии на тези заболявания, включително допълнителни изследвания, които са нужни за диференциална диагноза на атипични/ранни/наследствени случаи, включващи изследвания за биомаркери, невроизобразяващи и генетични фактори. Центъра е член **на** European Multisystem Atrophy Study Group и Movement Disorder Society.

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Association of an extended haplotype in the *tau* gene with progressive supranuclear palsy

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We describe two extended haplotypes that cover the human *tau* gene. In a total of ~200 unrelated caucasian individuals there is complete disequilibrium between polymorphisms which span the gene (which covers ~100 kb of DNA). This suggests that the establishment of the two haplotypes was an ancient event and either that recombination is suppressed in this region, or that recombinant genes are selected against. Furthermore, we show that the more common haplotype (H1) is significantly over-represented in patients with progressive supranuclear palsy (PSP), extending earlier reports of an association between an intronic dinucleotide polymorphism and PSP.

INTRODUCTION

Progressive supranuclear palsy (PSP) is the second most frequent cause of degenerative parkinsonism after Parkinson's disease (PD) (1). In addition to parkinsonism, the clinical symptoms include early postural instability and supranuclear gaze palsy (2). Neuropathologically, PSP is characterized by abundant neurofibrillary tangles (NFTs) and neuropil threads consisting of hyperphosphorylated Tau protein. The tangles observed in PSP differ in both distribution and composition from those associated with Alzheimer's disease (AD). In PSP, the tangles are primarily localized to subcortical regions and are found in both neurons and glia, whereas in AD they are more widespread, largely cortical and are limited to neurons. At the ultrastructural level the filaments that make up the tangles in PSP are straight, in contrast to the paired helical filaments (PHFs) that are the most abundant species associated with the tangles in AD (3,4).

There are six major protein isoforms of Tau in the adult human brain (Fig. 1). These are generated by alternative splicing of exons 2, 3 and 10 (5). Exons 9–12 encode four microtubule binding domains that are imperfect repeats of 31 or 32 residues (6). Alternative splicing of exon 10 generates isoforms with either four (exon 10+; '4-repeat Tau') or three (exon 10–; '3-repeat Tau') microtubule binding domains (7). The NFTs consisting of straight filaments that are observed in PSP contain only 4-repeat Tau isoforms, whereas the NFTs consisting of PHFs found in AD contain all six major Tau isoforms (4-repeat and 3-repeat) (4).

Mutations in the *tau* gene have recently been found to be associated with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (8–10). In this context, we have recently shown that mutations in the 5′ splice site of *tau* exon 10 increase the incorporation of this exon into *tau* mRNA (9) and thus increase the proportion of 4-repeat Tau isoforms (10). In affected families this increase is associated with the formation of 4-repeat Tau NFTs and leads to frontotemporal dementia. This observation shows that the control of this alternative splice event is critical and that disregulation can result in tangle formation and neurodegeneration (9–11).

The genetics of PSP had not been studied in great detail until recently, as the disease was usually considered to be sporadic in nature. However, Conrad *et al.* (12) demonstrated an association between a polymorphic dinucleotide marker, found between exon 9 and exon 10 of the *tau* gene, and PSP. This initial result has subsequently been confirmed by at least four other studies (13–16). In each case, an over-representation of the most common allele (a0) and genotype (a0a0) in the PSP group was reported. However, due to the nature of the dinucleotide polymorphism it was considered unlikely that this variation was biologically significant in the disease process, but was in fact in disequilibrium with other polymorphisms (12). The identification of mutations in the *tau* gene that influence alternative splicing of exon 10 and lead to the development of FTDP-17 (9,10) has led to speculation that the location of the dinucleotide polymorphism (between exons 9 and 10) might be significant and an indication that if the polymorphism itself was not functionally relevant, the critical variation would be nearby.

During the sequencing of the *tau* gene in FTDP-17 cases, we and others identified a series of polymorphisms scattered through the gene (8,9). Here we investigate the extent of disequilibrium between these polymorphisms and their association with PSP as a step towards defining the precise mechanism of genetic susceptibility for PSP at the *tau* locus.

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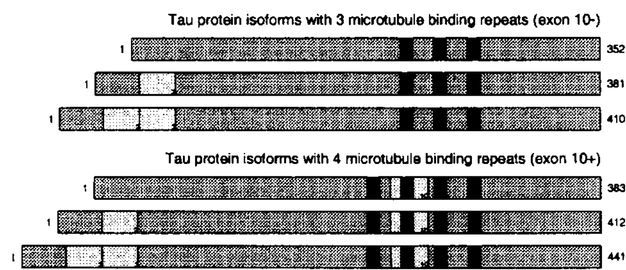

Figure 1. The six major isoforms of Tau. Regions encoded by alternatively spliced exons 2, 3 and 10 are indicated by light shaded boxes. Microtubule binding repeats encoded by exons 9–12 are indicated by vertical black bars.

RESULTS

Identification of extended haplotypes in the tau gene

Sequence analysis of the coding region and flanking intronic sequences in the *tau* gene, primarily in FTDP-17 families (8,9), identified a series of single nucleotide polymorphisms (SNPs) in exons 1, 2, 3, 9 (three polymorphisms), 11 and 13 (Table 1) which were present in both controls and patients. Analysis of the occurrence of these polymorphisms (Table 2) revealed that they are in complete disequilibrium with each other ($P < 1 \times 10^{-100}$). Thus, there are two extended haplotypes (designated H1 and H2) that cover the entire *tau* gene (~100kb; Fig. 2). We did not identify a single recombinant event within these haplotypes in any of the unrelated patient and control individuals tested (>200 total). The absence of recombinant events within the *tau* gene in this large group of unrelated individuals implies strongly that these haplotypes were established early in the history of the Caucasian population tested. One rare SNP (SNP 9iii in Tables 1 and 2) in exon 9 is present only on the more common H1 haplotype, suggesting that it arose by an independent mutagenic event after the establishment of this haplotype. In addition, the dinucleotide polymorphism (a0), which had been shown previously to be associated with PSP, is also inherited with the two extended haplotypes: dinucleotide polymorphism alleles a0 (11 repeats), a1 (12 repeats) and a2 (13 repeats) are inherited with the H1 haplotype, whereas the a3 (14 repeats) and a4 (15 repeats) alleles are inherited with the H2 haplotype. Given that a0 and a3 are the most common alleles (70.5 and 23%, respectively, in our control individuals; Table 3), it seems highly likely that a0 and a3 were present when the extended haplotypes were established, and that a1, a2 and a4 arose from subsequent slippage events (Fig. 2). This observation provides further testament to the conservation and antiquity of the two haplotypes.

Association of the extended *tau* haplotypes with PSP

We tested each of the polymorphisms (SNP and dinucleotide) and, thus, the extended haplotypes for association with PSP. A total of 65 cases of PSP were employed in this analysis (mean age 65.3 years); 18 were autopsy-confirmed cases while the remainder conformed to National Institute of Neurological Disorders and Stroke (NINDS) criteria for probable PSP. Aged caucasian controls ($n = 135$, mean age 63 years) were used for comparison.

Table 1. Details of SNPs

SNP	Primer sequence and name		Product (bp)	Digestion enzyme	Digestion products	
					Allele A (bp)	Allele B (bp)
1	CAACACTCCTCAGAACTTATC	1F	229	*AluI*	229	183 and 46
	CAGTGATCTGGGCCTGCTGTG	1R				
2	CAGCTCCACAGGACACTGCTC	2F	298	*BsaHI*	201 and 97	298
	GGAGTGAGCACATCTCTCAG	2R				
3	GGGCTGCTTTCTGGCATATG	3F	297	*BanII*	165 and 132	165, 68 and 64
	CCTCACTTCTGTCACAGGTC	3R				
9i	CCACCCGGGAGCCCAAGAAGGTGCC	9iF[a]	152	*MspI*	147 and 5	128, 19 and 5
	CTGGTGCTTCAGGTTCTCAGTG	9iR				
9ii	CGAGTCCTGGCTTCACTCC	9F	370	*MaeII*	370	127 and 243
	CTTCCAGGCACAGCCATACC	9R				
9iii	CGAGTCCTGGCTTCACTCC	9F	370	*BstNI*	257, 54, 26, 20 and 6	201, 56, 54, 26, 20 and 6
	CTTCCAGGCACAGCCATACC	9R				
11	GCTCATTCTCTCTCCTCCTC	11F	173	*AluI*	173	24 and 149
	CCAGGACTCCTCCACCCCATGCAGC	11R[a]				
13	ACTTCATCTCACCCTCCCTC	13F	597	*Tsp509I*	276, 94, 78, 72, 62 and 15	370, 78, 72, 62 and 15
	CCTCTCCTTCTCCCTCTTCTAC	13R				

[a]Mismatch primer sequence, designed to create an artificial restriction site. SNP numbering denotes the exon in which each is observed. SNPs were originally identified through sequence analysis of the *tau* gene primarily in FTDP-17 families (8,9).

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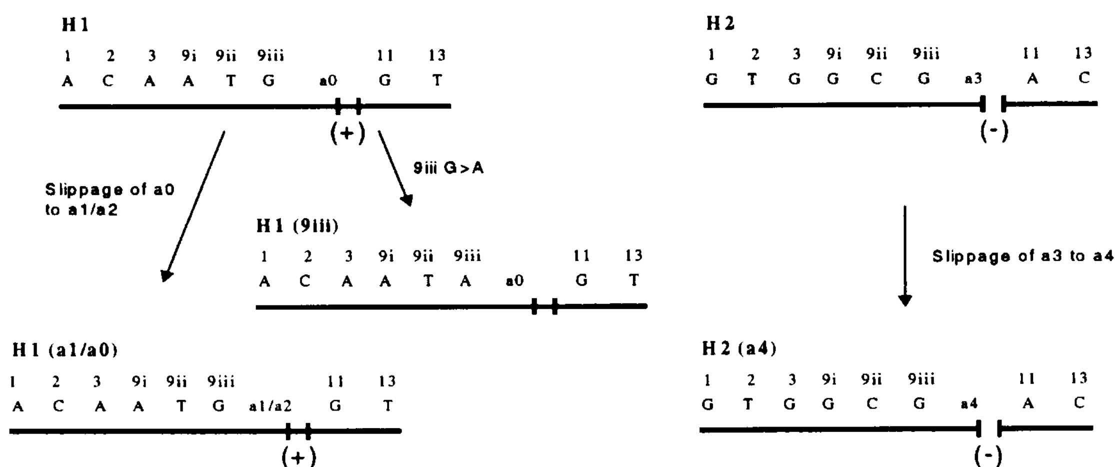

Figure 2. Human *tau* haplotypes. Schematic representation of the development of various human *tau* gene haplotypes. Ancestral haplotypes H1 and H2 are modified by subsequent mutational events (slippage of the dinucleotide polymorphism and the appearance of the exon 9iii polymorphism) but are not altered by recombination. SNPs that were typed in this study are shown at points along the *tau* gene with the nucleotide present in each haplotype indicated. The 238 bp deletion between exons 9 and 10 is shown by a break in the gene in the H2 haplotype. The presence or absence of this region is denoted by (+) or (–), respectively.

Table 2. Genotype frequencies of the common *tau* SNPs in PSP and control series

SNP	Genotype frequency % (number of individuals with each genotype)						χ^2	*P*-value
	H1 H1		H1 H2		H2 H2			
	PSP	Control	PSP	Control	PSP	Control		
exon 1	88.9 (56)	62.8 (91)	11.1 (7)	30.3 (44)	0	6.9 (10)	15.214	0.0005
exon 2	87.3 (55)	62.8 (91)	12.7 (8)	31.0 (45)	0	6.2 (10)	13.944	0.0009
exon 3	88.0 (44)	62.2 (89)	12.0 (6)	30.8 (44)	0	7.0 (10)	12.102	0.0024
exon 9i	88.5 (54)	63.8 (74)	11.5 (7)	30.2 (35)	0	6.0 (7)	12.952	0.0015
exon 9ii	88.3 (53)	63.0 (92)	11.7 (7)	30.8 (45)	0	6.2 (9)	13.753	0.0010
exon 9iii[a]	95.0 (57)	91.0 (131)	5.0 (3)	9.0 (13)	0	0	0.951	0.6217
exon 11	88.4 (38)	64.4 (87)	11.6 (5)	28.9 (39)	0	6.7 (9)	9.456	0.0088
exon 13	88.7 (47)	63.3 (83)	11.3 (5)	29.8 (39)	0	6.9 (9)	12.317	0.0021

[a]9iii is a rare polymorphism that occurs only on the H1 haplotype. The number of individuals typed with each genotype for individual polymorphisms is given in parentheses. Chi-squared analysis testing association between individual *tau* polymorphism genotypes and PSP is presented. H1 allele frequencies (not shown) also displayed a significant association with PSP.

Table 3. Allele and genotype frequencies of the intronic dinucleotide polymorphism in PSP cases and in controls

		PSP (n = 64)	Controls (n = 139)
Allele frequency % (number of chromosomes)	a0	89.8 (115)	70.5 (196)
	a1	4.7 (6)	5.7 (16)
	a2	0	0.4 (1)
	a3	5.5 (7)	23 (64)
	a4	0	0.4 (1)
		$\chi^2 = 20.83$, $P = 0.00034$ (4 df)	
Genotype frequency % (number of individuals)	a0/a0	79.7 (51)	51.1 (71)
	a0/a1	9.4 (6)	10.1 (14)
	a0/a3	10.9 (7)	28.1 (39)
	a0/a4	0	0.7 (1)
	a1/a3	0	1.4 (2)
	a2/a3	0	0.7 (1)
	a3/a3	0	7.9 (11)
		$\chi^2 = 18.56$, $P = 0.00497$ (6 df)	

Chi-squared analysis testing association between *tau* a0 haplotypes and genotypes and PSP is presented.

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Table 4. Extended haplotype and genotype frequencies in PSP and the aged control series

		PSP (n = 64)	Controls (n = 145)
Haplotype	H1	93.7% (120)	78.4% (228)
	H2	6.3% (8)	21.6% (62)
		χ^2 = 14.58, P = 0.00013 (1 df)	
Genotype	H1 H1	87.5% (56)	62.8% (91)
	H1 H2	12.5% (8)	31.0% (45)
	H2 H2	0	6.2% (9)
		χ^2 = 13.85, P = 0.00098 (2 df)	

For analysis of the extended haplotypes, all cases in which two or more of the SNPs had been scored were used, thus for this series more samples (for PSP n = 64) were available. Chi-squared analysis of association between *tau* extended haplotypes and genotypes and PSP is presented.

Initial analysis focused on the dinucleotide TG repeat polymorphism that had previously been shown to be associated with PSP. A significant over-representation of the most common allele (a0) and genotype (a0a0) was observed in the PSP cases compared with controls (Table 3). A proportion of the cases (n = 24) in this study had previously been used to demonstrate the association of this polymorphism with PSP (14); therefore, this observation was anticipated.

In addition, each of the polymorphisms displayed evidence of association with PSP in that the most common allele (H1) and genotype (H1H1) were significantly over-represented in this group compared with controls (Table 2). Since each of these SNPs are in complete disequilibrium with each other in this population, we also analyzed the data for association between each extended haplotype and PSP (Table 4). Again, a significant association with PSP was observed with the most common haplotype [H1, χ^2 = 14.58, P = 0.00013, 1 degree of freedom (df)] and genotype [H1H1, χ^2 = 13.85, P = 0.00098, 2 df). The odds ratio, for developing PSP, with the inheritance of the H1H1 genotype was calculated to be 4.08 [8.79 > CI (95%) > 1.89].

Sequence analysis of *tau* in PSP cases

Exons 9–13 were sequenced in 60 of the PSP cases employed in the association study, 13 of which were autopsy confirmed. These exons were analyzed as all of the mutations identified in *tau* associated with FTDP-17 have been found in this region of the gene. No missense or exon 10 5′ splice site mutations were identified in any of 60 PSP cases sequenced, indicating that typical PSP is not caused by similar mutations to those observed in FTDP-17 families. In 27 of these PSP cases (six confirmed by autopsy), the entire coding region of the *tau* gene was analyzed; again, no mutations were identified.

In addition to sequencing the coding exons, we also examined ~1 kb of intronic sequence 5′ and 3′ of *tau* exon 10. The reason for this analysis is that we consider the region around exon 10 to be the most likely candidate for biologically relevant genetic variation. This is because the tangles in PSP consist of 4-repeat Tau isoforms and when similar inclusions are observed in FTDP-17 families they are associated with missense or splice site mutations in exon 10 (4,9,11,17,18).

Sequence analysis of the introns flanking *tau* exon 10 revealed the presence of a deletion, positioned between –951 and –713 nucleotides upstream of exon 10. This 238 bp deletion is inherited as part of the less common H2 extended haplotype and thus shows a negative association with PSP.

DISCUSSION

We identified a series of eight common SNPs in the *tau* gene and found that these were inherited in complete disequilibrium with each other and with the dinucleotide polymorphism. Together, these polymorphisms define two extended haplotypes that contain at least the entire *tau* gene (exons 1–13; ~100 kb) and have remained uninterrupted by recombination in all the unrelated individuals examined in this study. These data clearly suggest that these two haplotypes were established early in the history of the Caucasian population. Indeed, additional polymorphisms have clearly arisen by independent mutational events rather than recombination since the establishment of the two haplotypes (point mutations and slippage of the dinucleotide polymorphism). The fact that there is little or no recombination over the entire length of the *tau* gene suggests that there must either be suppression of recombination in this chromosomal region, or selection against recombinant alleles.

Our association studies have demonstrated that there is a significant over-representation of the more common (H1) haplotype and genotype in PSP cases compared with controls. Thus, the earlier reports (12–16) of an association with the most common allele of the dinucleotide polymorphism (a0), between exon 9 and 10, are a reflection of this association with the broader haplotype. Indeed, using the results from the association study alone, it is not possible to determine any information about the position of the biologically relevant polymorphism that directly influences the risk of developing PSP, since the extended haplotype contains all of the *tau* gene analyzed.

From a biological perspective, there are three possibilities that might explain the association between *tau* and PSP: (i) there may be crucial differences between the two haplotypes in terms of the expression of the Tau protein; (ii) there may be differences in the splicing of the two cognate proteins; or (iii) a pathogenic, but non-coding, mutation has occurred on the H1 background. The fact that the brains of patients with PSP contain NFTs that consist of Tau isoforms with four microtubule binding repeats (4) suggests that there may be disruption of exon 10 alternative splicing in brain regions affected by PSP. Therefore, it would seem that the best candidate for a role in the pathogenesis in PSP is genetic variability that affects the alternative splicing of *tau* exon 10. In order to search for such variability we sequenced 1 kb into each intron flanking exon 10 and located a 238 bp deletion. It is possible that the presence or absence of the deleted region influences the alternative splicing of exon 10 in a neuron-specific manner, thus affecting the risk of developing PSP. Clearly, further studies, such as splicing analysis, will be needed to determine the role of *tau* exon 10 alternative splicing in the development of PSP and, thus, the significance, if any, of this deletion.

The association of polymorphisms in *tau* with PSP demonstrates that Tau dysfunction is probably crucial to the development of this disease. However, it remains to be determined if Tau dysfunction is the primary lesion in the pathogenesis of PSP or if some other initiating event is required first and that variability in the *tau* gene simply influences the sensitivity of specific neurons and glia to this initial insult.

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MATERIALS AND METHODS

Patient ascertainment

All patients used in this study were Caucasian. Individuals were diagnosed with probable PSP utilizing guidelines set by the NINDS (2). These criteria have been reported to have an optimal specificity of 100% (2). Neuropathologic findings in 18 cases were also consistent with PSP (NINDS criteria).

PCR and sequence analysis

Tau exons (1–5,7,9–13) were amplified from genomic DNA from individuals with primers designed to flanking intronic sequence (8,9,19). Exons 4A, 6 and 8 are essentially absent in Human *tau* brain mRNA and were therefore not analyzed (19). Twenty-five nanograms of DNA were used in a 50 µl reaction mixture containing 20 pmol of each primer, 0.2 mM dNTPs, 1 U Taq Gold polymerase (Perkin Elmer, Foster City, CA), 1.5 mM $MgCl_2$, 75 mM Tris–HCl, pH 9.0, 20 mM $(NH_4)_2SO_4$ and 0.01% Tween-20. Amplification of exon 9 required the addition of 5% DMSO. Amplifications were performed oil-free in Hybaid Touchdown thermal cyclers (Hybaid, Cambridge, UK). Conditions were 35 cycles of 94°C for 30 s, 60 to 50°C touchdown annealing for 30 s, and 72°C for 45 s with a final extension of 72°C for 10 min. All products were purified using the Qiaquick PCR Purification kit (Qiagen, Chatsworth, CA). For each exon, 100 ng of product was sequenced in both directions using the Big Dye kit (Perkin Elmer) and relevant PCR primers. Sequencing was performed on an ABI377 automated sequencer and processed using Factura and Sequence Navigator software (Perkin Elmer).

Genotyping and polymorphism analysis

Previously reported polymorphisms (8,9) were analyzed by PCR amplification followed by digestion of the product with the diagnostic restriction enzyme (PCR–RFLP). For polymorphisms 9i and 11, where the polymorphic site did not alter an enzyme recognition site, a mismatch primer was designed to create an artificial site that could be used for genotyping (Table 1). In the PSP series, the genotypes in exons 9 and 11 were determined from the sequence analysis. The presence of the intronic 238 bp deletion was determined by visualizing PCR product on an agarose gel. PCR conditions were as previously described, using primer sequences GGAAGACGTTCTCACTGATCTG (sense) and AGGAGTCTGGCTTCAGTCTCTC (antisense). All samples were genotyped for the intronic dinucleotide repeat polymorphism by PCR using a tet-labeled forward primer, followed by analysis on the ABI377 using Genotyper software (Perkin Elmer). Frequency of polymorphisms are shown in Tables 2 and 3. Linkage disequilibrium between loci was determined by the 'Estimated Haplotype Frequencies' program (20).

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REPORT

Identification of a Novel Risk Locus for Progressive Supranuclear Palsy by a Pooled Genomewide Scan of 500,288 Single-Nucleotide Polymorphisms

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To date, only the H1 *MAPT* haplotype has been consistently associated with risk of developing the neurodegenerative disease progressive supranuclear palsy (PSP). We hypothesized that additional genetic loci may be involved in conferring risk of PSP that could be identified through a pooling-based genomewide association study of >500,000 SNPs. Candidate SNPs with large differences in allelic frequency were identified by ranking all SNPs by their probe-intensity difference between cohorts. The *MAPT* H1 haplotype was strongly detected by this methodology, as was a second major locus on chromosome 11p12-p11 that showed evidence of association at allelic ($P < .001$), genotypic ($P < .001$), and haplotypic ($P < .001$) levels and was narrowed to a single haplotype block containing the DNA damage-binding protein 2 (*DDB2*) and lysosomal acid phosphatase 2 (*ACP2*) genes. Since DNA damage and lysosomal dysfunction have been implicated in aging and neurodegenerative processes, both genes are viable candidates for conferring risk of disease.

Progressive supranuclear palsy (PSP [MIM 601104]) is the second-most-common form of parkinsonism, with a population prevalence rate of 6–6.4 per 100,000.[1,2] Clinical features include vertical-gaze palsy and postural instability.[3,4] PSP is characterized neuropathologically by neuronal and glial inclusions composed of aggregated microtubule associated protein tau (MAPT) in the basal ganglia and brain stem.[5,6] Mutations in the *MAPT* (MIM 157140) gene have been identified in patients with a clinical presentation of PSP.[7–14] A recent report also described linkage to chromosome 1q31.1 in a family with autosomal dominant PSP.[15] However, only the *MAPT* locus has been consistently associated with increased risk for idiopathic PSP.[16–20] The *MAPT* locus exists as two major haplotype groups, termed "H1" and "H2"[16] in European populations, with the H2 haplotype defined by >100 SNPs that are inherited in strong linkage disequilibrium (LD) with each other, reflecting the total absence of H1-H2 recombination.[21] Inheritance of two copies of the H1 haplotype (H1/H1) is a major genetic risk factor for PSP.[16] A large collection of pathologically confirmed PSP samples was used recently to fine map PSP risk on H1 chromosomes in PSP cases and controls. [22,23] PSP risk was associated with an extended subhaplotype, and narrowing the region for PSP risk to a 22-kb region in intron 0 of *MAPT* was accomplished by examining younger patients with, presumably, a larger genetic component to their disease.[22,23] The most likely explanation of the association with the *MAPT* H1 haplotype and PSP is that variants in the H1 (and H2) haplotypes confer risk of (protect against) disease by altering expression at the locus, with the risky H1 haplotypes expressing higher levels of *MAPT*.[22–26]

Calculations of population-attributable risk suggest that only ~68% of the risk of PSP can be accounted for by the *MAPT* H1 haplotype, suggesting there may be additional risk genes involved in PSP. We hypothesized that additional genetic loci involved in conferring risk of PSP could be identified through genomewide association (GWA) methods. The cost of performing an association study that involved individual genotyping of thousands of SNPs for a series this size was prohibitive, so, instead, we used a pooled-DNA approach to identify additional risk factors. Whereas a pooling-based genomewide scan of thousands of SNPs has been proposed in principle, in large part, these studies have not been used for the discovery of genes pre-

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Table 1. Predicted Allelic Frequencies for the Top 1,000 SNPs

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disposing to complex diseases,[27,28] likely because of technical concerns or lack of technology and analysis tools.

The patients used in the initial pooling study, the "original" series, were largely derived from pathologically confirmed subjects collected by the PSP Society and sent to D.W.D. for brain autopsy. As described elsewhere, the patient samples in this brain bank were donated from the United States and Canada.[29] The patient series is similar to the one that we employed in previous studies to fine map the H1 genetic risk,[22] with 288 subjects with a primary pathological diagnosis of PSP used to create the pool of PSP-affected patients. A total of 344 age- and sex-matched cognitively normal control individuals were obtained through the Normal and Pathological Aging protocol at the Mayo Clinic (Scottsdale),[30,31] to create the pool of control individuals. All patient and control individuals were white from the United States and Canada, and institutional review board (IRB)–approved protocols were used in the collection of all samples.

Replicate pools of patients with PSP and control individuals were created as described elsewhere.[32] Samples were genotyped on 20 replicate Affymetrix 500K arrays and 20 Affymetrix 100K, in accordance with the Affymetrix protocols, whereby each of the five replicate pools was genotyped on two replicate arrays. This design therefore yielded probe-intensity data for both platforms on 10 replicate arrays per cohort. Data were analyzed using GenePool software (TGen Bioinformatics Research Unit).[32] In brief, probe-intensity data were directly read from cell-intensity (CEL) files, and relative allele signal (RAS) values were calculated for each quartet. These values yield independent measures of different hybridization events and are consequently treated as individual data points. We

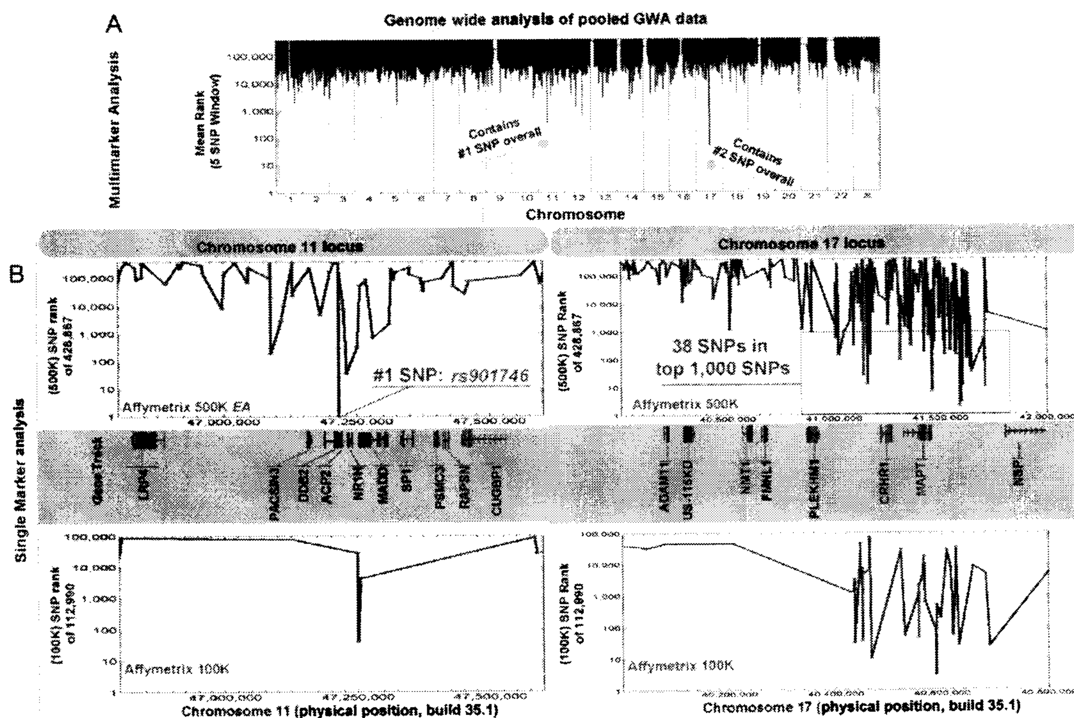

Figure 1. Two loci showing strong support for association by pooled analysis. *A*, Genomewide plot of the mean rank of five consecutive SNPs, calculated to identify clusters of high-ranking SNPs. The single best region was on chromosome 17, neighboring *MAPT*, and the second best region was on chromosome 11p12. Chromosome 11p12 also harbored the SNP that ranked #1 overall by single-marker statistics. *B*, Single-marker rank statistics for SNPs over the *MAPT* (*left*) and *DDB2/ACP2* (*right*) loci. SNPs deemed less reliable or showing high variability among replicates were removed, and the remaining SNPs were ranked in order from 1 (showing the greatest difference between cases and controls) to 428,867 (showing the least difference between cases and controls) with use of a silhouette-test statistic in GenePool software (TGen Bioinformatics Research Unit). Rank scores are plotted versus chromosomal position. Genes within the plotted chromosomal region are shown below. SNPs on the Affymetrix 500K platform are shown above, and SNPs on the Affymetrix 100K platform are shown below. EA = Early Access.

used a silhouette statistic to rank all genotyped SNPs,[35] because it avoids introducing unnecessary variance by averaging probe-intensity data from probes with different hybridization properties. Silhouette scores range from 1, where significant separation between data points has been achieved and cluster assignment can be made with confidence, to −1, where differences in allelic frequencies are less reliable. Poorly performing SNPs were identified by Affymetrix as unreliable in the transition to Mendel3 libraries or exhibited high variance between replicate arrays and were removed from the analysis; 428,867 SNPs remained. SNPs were ranked on the basis of silhouette score, whereby the SNP with the highest score was ranked 1 and the SNP with the lowest score was ranked 428,867, with use of Affymetrix's Mendel3 libraries for the Affymetrix 500K arrays and *Hin*dIII and *Xba*I libraries for the Affymetrix 100K arrays, then were sorted by chromosome and physical position. With this ranking, it is assumed that SNPs approaching a rank of 1 will have larger differences in allelic frequency. With each sample ranked by silhouette score, we calculated a sliding-window statistic of the mean rank for consecutively neighboring SNPs across a fixed window size. Window sizes from 2 to 31 were used.

Since the *MAPT* H1 haplotype is associated with disease with a haplotypic odds ratio (OR) of ~3–4,[16,22,23,34] it served as an internal positive control for the study. For analysis, we used the 500K data to identify chromosomal regions of interest (i.e., those with small mean-rank scores). The 100K data were then used to confirm that a region identified in the 500K analysis contained SNPs with large allelic frequency differences. The SNP with the single best statistical rank on the 500K chip was *rs901746* on chromosome 11p12, and the second-best SNP was *rs17662235*, near *MAPT*. The top 1,000 SNPs, based on individual statistical rank, are given in table 1. Multimarker statistics also identified both chromosome 11p12 and chromosome 17q21 (*MAPT*) regions with sliding windows of multiple sizes. Although we recognize that this type of statistic is biased because of genomewide LD, it allowed us to identify clusters of high-ranking SNPs that neighbor one another, which reduced the possibility of technical errors influencing the results. Shown in figure 1*A*, the *MAPT* locus, labeled as having the #2 SNP overall, showed the greatest evidence of differences between case and control pools with use of the sliding-window analysis, largely because of 38 SNPs within the top 1,000 SNPs overall and a total of 75 SNPs in the region with a rank score of <10,000 (fig. 1*B* and table 1). Examination of the individual SNPs with high rank scores over this locus showed SNPs that were derived from a region covering the full extent of the *MAPT* H1 haplotype, spanning nearly 1 Mb (fig. 1*B*).[36] All of the 75 SNPs with genotype-frequency data in the database resembled *MAPT* H2 variants (which differentiate between H1 and H2 *MAPT* haplotypes) rather than H1 variants (which differentiate between H1 subhaplotypes); this is because, in white populations, the SNP minor-allele frequency was ~0.2, whereas the minor allele of the SNP was absent or rare in Asian populations and African populations.[36,37] In addition, two of the SNPs with low rank

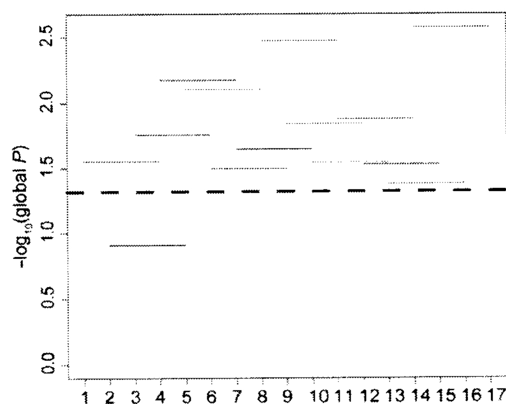

Figure 2. Haplotype sliding window–analysis results. The haplotype score–based method of Schaid et al.[40] was used to investigate evidence of association of haplotypes with case-control status. Only haplotypes with an estimated overall frequency of ⩾5% were considered for these analyses. Reported *P* values are based on asymptotic assumptions but were verified by simulating *P* values derived from 1,000 permutations of case and control labels and were found to be consistent. Global *P* values for each 4-marker haplotype are denoted as lines at the $-\log_{10}P$. Only young pathologically confirmed PSP cases (death at age <76 years) were used for the analysis. All individuals in the control group were used in all analyses, since no single SNP showed significantly different allelic frequency distribution in controls when stratified by age. Global $P = .01$ is denoted by a dashed line. SNP numbers are as noted in table 3.

Table 2. Association Analysis of *rs901746* in Original and Replication Series

		No. (%) of					GG versus AG and AA		
		Alleles		Genotypes					
Population	*n*	A	G	AA	AG	GG	OR	95% CI	*P*
Control combined	**735**	**1,011 (75)**	**335 (25)**	**377 (56)**	**257 (38)**	**39 (6)**	...	...	...
Control original	344	438 (78)	126 (22)	166 (58)	106 (37)	10 (4)	...	...	...
Control replication	391	573 (73)	209 (27)	211 (54)	151 (39)	29 (7)	...	...	...
PSP combined	**501**	**661 (68)**	**317 (32)**	**231 (47)**	**199(41)**	**59 (12)**	**2.2**	**1.5–3.4**	**.0001**
PSP original	288	374 (68)	178 (32)	131 (47)	112 (41)	33 (12)	4.0	1.9–8.3	.0001
PSP replication	213	287 (67)	139 (33)	100 (47)	87 (41)	26 (12)	1.7	1.0–3.0	.05

Table 3. Single-Marker Analysis of Tag SNPs in the Combined Series and in Both Young and Old Patient Populations

tagID[a] (SNP), and Allele	No. (%) of Alleles in Controls (n = 532)	All Cases (n = 448)		Young[b] Cases (n = 162)		Old[b] Cases (n = 182)	
		No. (%) of Alleles	P	No. (%) of Alleles	P	No. (%) of Alleles	P
1 (*rs11039130*):			**.003**		**.02**		.25
C	600 (69)	614 (75)		224 (76)		245 (72)	
T	274 (31)	202 (25)		72 (24)		95 (28)	
2 (*rs4647709*):			.5		.57		.88
C	806 (91)	787 (90)		292 (90)		331 (91)	
T	78 (9)	85 (10)		32 (10)		31 (9)	
3 (*rs2291120*):			**.0004**		**.003**		**.006**
T	781 (92)	859 (87)		280 (86)		317 (87)	
C	67 (8)	115 (13)		44 (14)		47 (13)	
4 (*rs10742797*):			.81		.72		.98
A	591 (81)	572 (81)		212 (82)		243 (80)	
T	143 (19)	134 (19)		48 (18)		59 (20)	
5 (*rs1685404*):			.72		.97		.96
G	598 (68)	560 (67)		213 (68)		237 (68)	
C	282 (32)	274 (33)		101 (32)		111 (32)	
6 (*rs7395581*):			.03		**.02**		.07
A	378 (71)	437 (65)		157 (63)		180 (65)	
G	152 (29)	233 (35)		93 (37)		96 (35)	
7 (*rs11039138*):			**.01**		**.02**		.37
G	470 (56)	442 (62)		168 (64)		173 (59)	
A	372 (44)	268 (38)		94 (36)		121 (41)	
8 (*rs2957873*):			.22		.52		.28
A	728 (83)	679 (81)		257 (81)		281 (80)	
G	150 (17)	163 (19)		59 (19)		69 (20)	
9 (*rs4647736*):			**.03**		**.04**		.12
C	807 (91)	736 (88)		273 (88)		305 (89)	
T	75 (9)	98 (12)		39 (13)		39 (11)	
10 (*rs2013867*):			**.004**		**.006**		.02
T	657 (74)	549 (66)		206 (66)		236 (67)	
C	229 (26)	279 (34)		106 (34)		114 (33)	
11 (*rs901746*):			**<.0001**		**.003**		**.004**
A	659 (76)	570 (67)		204 (65)		242 (68)	
G	213 (24)	282 (33)		110 (35)		116 (32)	
12 (*rs1050244*):			.53		.89		.44
C	851 (97)	823 (96)		307 (97)		343 (96)	
T	29 (3)	33 (4)		11 (3)		15 (4)	
13 (*rs11039143*):			.87		.64		.52
T	830 (98)	782 (98)		293 (98)		319 (98)	
G	18 (2)	16 (2)		5 (2)		5 (2)	
14 (*rs7118396*):			.16		.27		.13
C	741 (86)	688 (84)		253 (84)		287 (83)	
T	117 (14)	132 (16)		49 (16)		59 (17)	
15 (*rs12577530*):			**.0009**		**.005**		**.04**
G	784 (88)	701 (82)		260 (82)		296 (84)	
C	106 (12)	149 (18)		58 (18)		58 (16)	
16 (*rs7114704*):			.01		.3		**.0006**
C	813 (93)	803 (96)		288 (95)		343 (98)	
T	61 (7)	35 (4)		16 (5)		7 (2)	
17 (*rs10501320*):			**<.0001**		**.003**		.16
G	609 (70)	641 (78)		230 (79)		255 (74)	
C	265 (30)	179 (22)		62 (21)		91 (26)	

NOTE.—Significant P values are shown in bold.

[a] Tag SNPs were chosen on the basis of the tagging algorithm in Haploview v3.32 software,[39] with the "Pairwise Tagging Only" option selected and the r^2 threshold set at .8.

[b] Subjects aged <76 years were classified as "young"; subjects aged ≥76 were classified as "old."

Table 4. SNP Discovery Results from Sequencing *DDB2* and *ACP2* in 18 Subjects with PSP

	Genotype at *DDB2* 5′→3′			Genotype at *ACP2* 3′→5′					
	Intron 8	Intron 9	3′ UTR	Intron 6		Exon 5	Intron 3		Exon 1
Sample	*rs326222*	***rs901746***	*rs1050244*	*rs11039146*	*rs2242261*	*rs10838677*[a]	ss68362654[b]	*rs4752973*	*rs2167079*[c]
1	GG	GG	CT	CT	AA	AG	AG	AG	AA
2	GG	GG	CC	CC	AA	GG	GG	AA	AA
3	GG	GG	CC	CC	CC	GG	GG	GG	AA
4	GG	GG	CC	CC	AC	GG	GG	AG	AA
5	GG	GG	CC	CC	AC	GG	GG	AG	AA
6	GG	GG	CC	CC	CC	GG	GG	GG	AA
7	GG	GG	CC	CC	AA	GG	GG	AA	AA
8	GG	GG	CT	CT	AA	AG	AG	AG	AA
9	AG	AG	CC	CC	AA	GG	GG	AA	AG
10	GG	GG	CC	CC	AC	GG	GG	AG	AA
11	GG	GG	CC	CC	AC	GG	GG	AG	AA
12	GG	GG	CC	CC	AC	GG	GG	AG	AA
13	AG	AG	CC	CC	AA	GG	GG	AA	AG
14	AG	AG	CC	CC	AA	GG	GG	AA	GG
15	AG	AG	CC	CC	AA	GG	GG	AA	AG
16	AG	AG	CC	CC	AA	GG	GG	AA	AG
17	AA	AA	CC	CC	AA	GG	GG	AA	GG
18	AA	AA	CC	CC	AA	GG	GG	AA	GG

[a] Encodes synonymous change L165L.
[b] No rs number; submitted to dbSNP.
[c] Encodes nonsynonymous change R29Q.

scores (*rs12150111* and *rs807072*) were identified definitively as *MAPT* H2 variants from prior *MAPT* genomic sequencing efforts.[22]

The chromosome 11p12 region that showed the highest rank SNP by single-marker statistics and multimarker sliding-window analysis was a novel locus and therefore was examined in greater detail (fig. 1*B*). The top overall ranked SNP, *rs901746*, a SNP in intron 9 of the DNA damage-binding protein 2 (*DDB2* [MIM 600811]) gene, was chosen for follow-up in the individual samples comprising the pooled DNA. A significant increase of 10% in the G allele frequency was seen in cases versus controls ($P = .0002$) (table 2). The SNP was then genotyped in a second U.S. series to confirm the association. This "replication" sample ($n = 161$) was made up of both pathologically confirmed ($n = 97$) and clinically defined PSP case individuals ($n = 64$), as described in Rademakers et al.[22] A total of 165 age- and sex-matched cognitively normal control individuals were obtained from the Normal and Pathological Aging Protocol at the Mayo Clinic (Scottsdale).[30,31] In addition, for the *rs901746* and *rs2167079* analysis, additional pathologically confirmed cases ($n = 41$) and clinically defined PSP case indivuals ($n = 22$) were genotyped, and 252 age- and sex-matched cognitively normal control individuals collected at Mayo Clinic Jacksonville were used as a second source of controls.[22] All case and control individuals in this set were white from the United States and Canada, and IRB-approved protocols were used in the collection of all samples.

When allele frequencies at *rs901746* were examined in the replication sample set, a 6% increase in the frequency of the G allele in subjects with PSP was observed; however, because of the smaller sample size, this allele frequency difference is borderline significant ($P = .05$). When genotype distributions were examined in both PSP case-control series, the frequencies were very similar, with an increase from 4% to 12% in the GG genotype in the original population and an increase from 7% to 12% in the replication set. The allelic frequency difference in both series is explained by an apparent doubling of the GG frequency in subjects with PSP compared with controls, suggesting that risk at this locus acts in a recessive manner. We explicitly tested dominant, recessive, and additive models at this locus, and the model that best fit the data was a recessive one ($P < .0001$). The OR for harboring an *rs901746* GG genotype versus all other genotypes in the original series was 3.7 (95% CI 1.2–3.9) and was 1.7 (95% CI 1.0–3.0) for the replication series. When these individuals in both of these series were combined and analyzed, the combined

Table 5. Association Analysis of *rs2167079* in the Combined Series

SNP and Allele	No. (%) of Alleles: All Controls ($n = 735$)	No. (%) of Alleles: All Patients ($n = 501$)	*P*
rs901746:			**<.0001**
A	1,011 (75)	661 (68)	
G	335 (25)	317 (32)	
rs2167079:			**.002**
G	918 (73)	598 (67)	
A	332 (27)	292 (33)	

NOTE.—Results include the additional cases and controls used in the replication series.

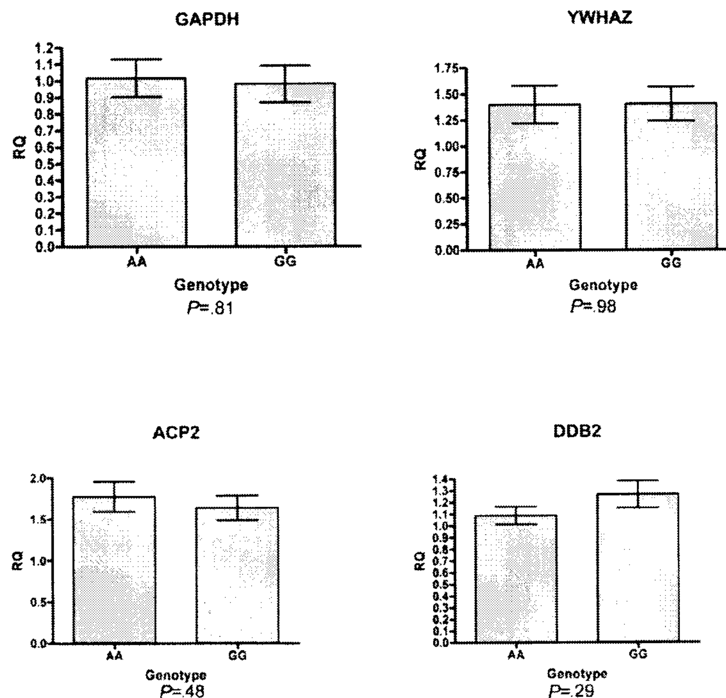

Figure 3. Relative mRNA expression with TATA-binding protein as an endogenous control. Plotted are relative levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH); tyrosine 3-monooxygenase/tryptophan 5-mono-oxygenase activation protein, zeta polypeptide (YWHAZ); ACP2 (assay Hs00155636_m1 [Applied Biosystems]); and DDB2 (assay Hs00172068_m1 [Applied Biosystems]) for 20 carriers of the *rs901746* AA (neutral) genotype and 20 carriers of the *rs901746* GG (risky) genotype. SE is denoted by the error bars. None of the comparisons between AA and GG carriers reach the level of statistical significance (*P* values noted below each graph). Similar results are seen when GAPDH or YWHAZ was used as the endogenous control (data not shown). RQ = relative quantity.

OR for the GG genotype compared with all other genotypes in the series was 2.2 (95% CI 1.4–3.4). To confirm that the *rs901746* association observed is not a control frequency artifact, we examined allele frequencies for *rs901746* in 250 cognitively normal controls recently published in a Parkinson disease (PD [MIM 168600]) GWA study.[38] We found that the frequency of the *rs901746* G allele in this independent control series was 0.27, consistent with our observed control frequencies (0.22 and 0.27).

The genomic context near *rs901746* was examined by downloading the CEPH-from-Utah SNP genotypes for 100 kb around *rs901746* from the HapMap genome browser and by examining the LD patterns and haplotype-block structure of the region with use of the Haploview software.[39] *rs901746* lies in the middle of a haplotype block encompassing at least two genes—the *DDB2* gene and the lysosomal acid phosphatase 2 (*ACP2* [MIM 171650]) gene—and can extend into the 3′ of another gene, nuclear receptor subfamily 1, group H, member 3 (*NR1H3* [MIM 602423]), depending on the type of haplotype-block definition used.[39] Variation in this 100-kb region could be fully described by 16 additional tag SNPs. These tag SNPs were genotyped in all PSP series, and both single and multimarker analysis was performed on the combined series (table 3). Single-marker analysis showed that nine tag SNPs showed significant allelic association. Of these, five tag SNP associations were highly significant (P values $\leq .003$), with *rs10501320* showing the greatest association after *rs901746* ($P < .0001$).

We examined the *DDB2/ACP2* tag SNP data set in different age groups in our combined series of cases and controls to see whether looking at younger cases might help further refine the associated region, as it had for the *MAPT* locus, where younger cases show a stronger association with the H1/H1 genotype.[22] Pathologically confirmed cases were divided into "young" and "old" groups on the basis of median age at death (75 years), and single-marker allelic association statistics were calculated using 2 × 2 contingency tables and were examined using χ^2 tests. On the whole, all of the SNPs that show significant association in the combined PSP case set also show significant association ($P < .05$) with the younger case subset, whereas there is less-significant association observed for the older cases.

To refine the disease-associated region, we performed haplotype-inference analysis in the young cases versus all controls, using a sliding-window approach.[40] As described elsewhere, this type of approach was key in refining the

associated region on the *MAPT* H1 haplotype.[22] However, as figure 2 displays, when data from all the tag SNPs were included in the analysis, there was no obvious resolution of the associated region when the young cases were considered separately. This may reflect the fact that the contribution to the overall signal of the association at this locus was not as great with the younger cases as had been seen with the *MAPT* locus; therefore, the sample size and/or the number of informative SNPs was inadequate to detect a smaller associated region.

Since a haplotype-inference approach was unsuccessful in narrowing the associated region, we decided to identify additional novel SNPs that may represent functional variant(s) accounting for increased risk of disease by sequencing a series of 18 subjects with PSP who had the various genotypes at *rs901746*, the majority of whom carried the risky GG genotype ($n = 11$ GG, $n = 5$ AG, and $n = 2$ AA) (table 4). Primers were designed to fully sequence coding exons of both *DDB2* and *ACP2*. Only one SNP, found 63 bp downstream of exon 3 in *ACP2*, was not already in the dbSNP database; however, this SNP appeared to be in near-complete LD with nearby *rs10838677* in exon 5 of *ACP2*, encoding a silent change (L165L). Interestingly, a number of SNPs identified through sequencing appeared to be in near-complete LD with *rs901746*, including *rs2167079*, a coding SNP in *ACP2* in which the minor allele changes the amino acid at position 29 from an arginine to a glutamine (R29Q). This converts the protein sequence to the mouse amino acid residue at the equivalent position. Interestingly, this position in ACP2 is predicted to encode the signal peptidase cleavage site,[41] suggesting that carriers of the minor allele encoding glutamine at position 29 may have altered cleavage of the signal peptide compared with those encoding arginine at that position. Since this SNP could affect function of the protein, we genotyped it through the combined series. Results from this analysis are shown in table 5. Overall, the LD between *rs901746* and *rs2167079* was high (cases $r^2 = 0.97$; controls $r^2 = 0.94$). As expected, significant allelic association was observed with *rs2167079* ($P = .002$); however, this was not any greater than the association observed with *rs901746*, suggesting that *rs2167079* is unlikely to fully explain the association at the *DDB2/ACP2* locus. We tested the R29Q variant for dominant, recessive, and additive models, and the additive model best fit the data ($P < .0001$).

In an alternative method for determining the gene responsible for disease risk at this locus, expression analysis was performed on the *DDB2* and *ACP2* genes. Analysis was performed, using real-time Taqman expression assays (Applied Biosystems), on mRNA extracted from the cerebella of 20 *rs901746* AA and 20 *rs901746* GG genotype carriers, to determine whether risk variants at the *DDB2/ACP2* locus have a direct effect on gene expression. Unfortunately, although DDB2 transcript levels are slightly increased in cases with a GG genotype, no significant differences were observed between the cases with AA and GG genotypes for either *DDB2* or *ACP2* mRNA levels (for *DDB2*, $P = .29$; for *ACP2*, $P = .48$) (fig. 3).

GWA studies are appealing because of their lack of bias, in that they represent a model-free approach for identification of new and novel genes that are involved in a disease process that may never be identified using other methodologies. However, even now, individually genotyping hundreds of individuals to perform a "traditional" GWA is not feasible for many rarer diseases, including PSP, because of the lack of available funding. Therefore, this type of pooled genomewide approach potentially represents a fast and economical initial solution to this problem. Pooling methods lack the analytical flexibility inherent in a traditional genomewide study because it is not possible to reanalyze the data with use of subgroups of cases or controls or to perform true haplotype-scanning analyses. However, there is still some uncertainty about how best to analyze the large amounts of individual genotype data used in GWA studies. An early GWA study of the PD showed problems in replication of results, potentially because of problems in study design.[42–47]

Although pooling methods clearly have limitations, the analysis procedures we used in the GenePool software (TGen Bioinformatics Research Unit) were developed using individual genotype data from samples that were also pooled, thereby allowing the algorithms to be adjusted until they predicted SNP ranks on the basis of what was known from the individual genotype data.[32] In the present analysis, we had prior knowledge that the *MAPT* H1 haplotype is associated with disease, so it could serve as a positive control for the genomewide analysis.

The identification of a new risk locus for PSP on chromosome 11 from the pooled genomewide approach was confirmed in a second U.S. PSP case-control series, with similar allele and genotype frequencies. Closer examination of this locus by dense SNP genotyping suggests that the association spans the entire haplotype block containing the *DDB2* and *ACP2* genes. Examination of potential functional variants yielded no definitive explanation for the observed association.

Both *ACP2* and *DDB2* are reasonable candidate genes that highlight previously implicated pathways for neurodegenerative disease. There are many lines of evidence suggesting a role for lysosomes and autophagic processes in neurodegeneration. Autophagy has been implicated in the clearance of protein aggregates, a common feature of many neurodegenerative disorders.[48,49] Interestingly, patients with lysosomal-storage disorders often exhibit neurological phenotypes with pathology similar to that seen in PSP.[50–52] Two lines of evidence implicate *ACP2* in neurodegeneration. First, it has been reported that, in brains of subjects with Alzheimer disease (AD [MIM 104300]), microglia surrounding the amyloid plaques stain strongly for ACP2.[53] In addition, cerebrospinal fluid from half of the examined subjects with AD showed evidence of ACP2 activity, whereas patients not affected with AD showed no activity.[53] These results leave open the question of whether

ACP2 in AD is just a secondary marker of neurodegeneration or perhaps plays a more active role in the neurodegenerative process. Second, knockouts and mutations of *Acp2* in mice have neurological phenotypes.[54,55] Neuropathology of $Acp2^{-/-}$ tissue showed increased lysosomal staining (as detected by lamp-1 and cathepsin D immunoreactivity), primarily in glial cells. Interestingly, ~7% of these $Acp2^{-/-}$ mice presented with generalized seizures after age 8 wk, and it has been suggested that this phenotype may be correlated with the defective lysosomal storage observed in glial cells.[54] The observation that loss of Acp2 causes deficits in glial lysosomal storage in the $Acp2^{-/-}$ mice may also be significant, given that, in PSP, there is abundant MAPT-inclusion pathology within glia (astrocytes and olgodendroglia), as well as in neurons.[56]

Mutations in the *DDB2* gene are responsible for xeroderma pigmentosum (XP) complementation group E (XPE [MIM 278740]). Interestingly, some mutations in the nucleotide excision–repair pathway that cause the diseases XP and Cockayne syndrome (MIM 216400) present with neurological phenotypes; however, XPE does not seem to be one of them.[57,58] DDB2 forms a ubiquitin E3-ligase complex, with DNA damage-binding protein 1 (DDB1 [MIM 600045]) and Cullin 4a (CUL4A [MIM 603137]), that binds damaged DNA. Both histone H2A (H2AA [MIM 603137]) and XP complementation group C (XPC [MIM 278720]) proteins have been implicated as substrates for the DDB1/DDB2/CUL4A complex upon activation.[59,60] Ubiquitination of histone H2A may change local chromatin configuration at the damage site, thereby allowing access to other DNA-repair proteins farther down the pathway.[60] The accumulation of damaged DNA in aging brain suggests that DNA-repair capacity is reduced as we age and appears to be selective to genes important in learning and memory. Interestingly, there is evidence of brain-specific alternatively spliced forms of *DDB2* that splice out either exons 4–7 or exons 4 and 6 alone.[61] The proteins encoded by these alternatively spliced transcripts act as dominant negative inhibitors of DNA repair, when tested in an in vitro system.[61] It will be interesting to tease apart which gene or genes at this locus are involved in conferring risk of PSP, but functional studies, rather than genetic ones, will probably be required to address these issues.

Given the size of the association seen at the *DDB2/ACP2* locus, the fact that the described PSP series represents the largest collection of PSP-affected subjects worldwide, and the fact that our U.S. replication series is underpowered to detect changes with an OR <2.0, we may be at the limit of what can be consistently detected and confirmed using the case-control populations available. Six additional weaker loci were identified in the genomewide screen that still need to be analyzed in detail, and it will be interesting to examine this potential power issue in closer detail. This genomewide analysis has identified a novel second locus implicated in PSP risk, accelerating research and the hope of identifying effective therapeutics for this devastating disease.

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Web Resources

The URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PSP, *MAPT, DDB2*, PD, *ACP2, NR1H3*, AD, XPE, Cockayne syndrome, DDB1, CUL4A, H2AA, and XPC)
TGen Bioinformatics Research Unit, http://bioinformatics.tgen.org/ (for the GenePool source code)

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ORIGINAL CONTRIBUTION

Familial Progressive Supranuclear Palsy

Detection of Subclinical Cases Using ^{18}F-Dopa and ^{18}Fluorodeoxyglucose Positron Emission Tomography

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Background: Progressive supranuclear palsy (PSP) is generally considered to be a sporadic disease; however, occasional cases of familial PSP have been described. The rarity of reports of familial PSP may be attributed in part to an inability to detect subclinical disease in affected relatives who subsequently die before symptoms clinically develop.

Objective: To study regional cerebral dopaminergic function and glucose metabolism in members of 2 large kindreds with familial PSP to identify subclinical cases.

Methods: Three clinically affected members from the 2 PSP kindreds were scanned with both ^{18}F-dopa and ^{18}fluorodeoxyglucose (^{18}FDG) positron emission tomography (PET). Fifteen asymptomatic first-degree relatives were scanned with ^{18}F-dopa PET; 10 of them also underwent a second PET study with ^{18}FDG.

Results: All 3 clinically affected PSP patients showed a significant reduction in caudate and putamen ^{18}F-dopa uptake along with a significant reduction in striatal, lateral, and medial premotor area and dorsal prefrontal cortex glucose metabolism. In 4 of the 15 asymptomatic relatives, caudate and putamen ^{18}F-dopa uptake was 2.5 SDs lower than the normal mean. These 4 subjects and a fifth asymptomatic relative with normal ^{18}F-dopa uptake showed a significant reduction of cortical and striatal glucose metabolism in a pattern similar to that of their affected relatives.

Conclusion: ^{18}F-dopa and ^{18}FDG PET allowed us to identify 5 cases with subclinical metabolic dysfunction among 15 subjects (33%) at risk for PSP, suggesting that this approach is useful for characterizing the pattern of aggregation in PSP kindreds.

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PROGRESSIVE supranuclear palsy (PSP) is a late-onset neurodegenerative disease characterized by supranuclear vertical gaze palsy, postural instability, rigidity, bulbar dysfunction, and dementia with the variable presence of pyramidal and cerebellar signs.[1,2] It is usually considered a sporadic disorder, even though a few familial, pathologically proven PSP cases have been reported.[3] In a recent article concerning PSP kindreds from Europe and North America, 12 probands with 22 secondary cases with typical clinical PSP features have been described.[4] Thus, the apparent rarity of familial PSP may reflect the difficulty in recognizing PSP cases in epidemiological surveys. In particular, atypical presentations of PSP cases may hinder accurate phenotypic assignment, and mortality owing to other diseases may be responsible for a censoring effect with subclinically affected relatives dying before symptoms develop.

Positron emission tomography (PET) has proven to be a reliable method for detecting in vivo subclinical dysfunction in degenerative diseases. ^{18}F-dopa PET studies have shown that 25% of asymptomatic adult relatives of patients with familial Parkinson disease (PD) and 55% of elderly asymptomatic co-twins of PD patients show subclinical dopaminergic nigrostriatal dysfunction[5,6]; ^{11}C-raclopride PET revealed that 50% of asymptomatic adult carriers of the Huntington disease *IT15* gene had significant reductions in striatal dopamine D_2 receptor binding.[7]

In this study we used ^{18}F-dopa and ^{18}fluorodeoxyglucose (^{18}FDG) PET to investigate regional cerebral dopaminergic function and glucose metabolism in clinically affected patients and their asymptomatic relatives from 2 kindreds with familial PSP. Our aim was to determine the prevalence of subclinical cases with disease.

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SUBJECTS AND METHODS

We studied 2 unrelated kindreds in which PSP was present across several generations. The members of these families were referred to our PET Centre from the Movement Disorder Clinics at the National Hospital for Neurology, Queen Square, London, England (kindred 1) and from the Fundacion Jimenez Diaz, Avenida de los Reyes Catolicos, Ciudad Universitaria, Madrid, Spain (kindred 2). One of the 2 original probands (kindred 2) had died by the time of this study, and postmortem analysis showed typical PSP neurofibrillary tangle disease. In the antecedent relatives, the diagnosis of PSP was based on review of hospital records.

The diagnosis of possible or probable PSP was made according to the National Institute of Neurological Disorders and Stroke PSP society criteria.[8] Three affected subjects (2 from kindred 1; 1 from kindred 2) had an akinetic-rigid syndrome poorly responsive to levodopa with onset at age 53, 60, and 70 years, respectively; 2 had a supranuclear down-gaze palsy, whereas the third had slowing of vertical saccades and prominent postural instability. All 3 PSP subjects had axial rigidity, and 2 had a pseudobulbar palsy. Two of the 3 patients had mild to moderate cognitive impairment of frontal type. (For more clinical details see reference 4.) To establish the pattern of dopaminergic and metabolic dysfunction in these families, we studied the 3 clinically affected members with ^{18}F-dopa and ^{18}FDG PET (**Table**).

All asymptomatic first-degree adult relatives aged 40 years or older were asked to participate in the study. Fifteen subjects agreed to undergo PET scanning with both ^{18}F-dopa and ^{18}FDG. All 15 relatives underwent ^{18}F-dopa PET, and 10 of them also underwent ^{18}FDG PET. Of the other 5 that did not have ^{18}FDG, 3 could not be rescanned for technical reasons, and 2 subjects refused to have a second scan because they experienced claustrophobia during the first scan.

The 15 asymptomatic relatives had no history of neurological illness and had not taken drugs known to affect the dopaminergic system. At the time of scanning, all underwent a full neurological examination. Fourteen subjects had no signs or symptoms of neurological disease, while 1 subject, aged 68 years (kindred 2, III.3), had an isolated postural hand tremor. The characteristics of these 15 subjects are detailed in Table 1B.

SCANNING PROTOCOLS

Permission for these studies was obtained from the Ethics Committee of the Hammersmith Hospitals Trust, London. Approval to administer radio-labeled ligands was obtained from the Administration of Radioactive Substances Advisory Committee of the United Kingdom. Written consent was obtained from all subjects after a full explanation of the procedure.

The PET studies were performed using a camera at the Medical Research Council, Cyclotron Building, Hammersmith Hospital, London (ECAT 953B; CTI Inc, Knoxville, Tenn). This camera acquired data simultaneously from 31 consecutive transaxial planes (slice separation, 3.4 mm with an average in-plane resolution of 6 mm full width at half maximum). Scanning was performed with the orbitomeatal line parallel to the detector rings. A 10-minute transmission scan, using a retractable ^{68}Ga/^{68}Ge ring source, was performed prior to the acquisition of the emission data to correct for tissue attenuation.

^{18}F-Dopa Scans

Prior to ^{18}F-dopa injection, subjects were given oral carbidopa, 100 mg, 1 hour before and 50 mg 5 minutes before the study to block peripheral aromatic amino acid decarboxylase. A mean dose of 4.4 mCi (163 MBq) of ^{18}F-dopa was infused into each subject intravenously over 30 seconds, and the dynamic emission data were acquired in 3-dimensional (D) mode as 25 time-frames over 95 minutes.

^{18}FDG Scans

A mean dose of 4.7 mCi (174 MBq) of ^{18}FDG was administered to each subject by intravenous infusion over 30

Continued on next page

RESULTS

CLINICALLY AFFECTED RELATIVES

^{18}F-Dopa

Mean ^{18}F-dopa caudate and putamen K_i values (0.0051±0.0011 min^{-1} and 0.0049±0.008 min^{-1}) were significantly reduced (P<.001) in the 3 PSP relatives compared with normal subjects (0.0104±0.0010 min^{-1} and 0.0102±0.009 min^{-1}) (**Figure 1**).

^{18}Fluorodeoxyglucose

The SPM analysis revealed areas of significantly reduced ^{18}FDG uptake in bilateral lateral and medial premotor areas (areas 6 and 8) (x, y, z=−24, −8, 56; z score, 5.31; and x, y, z=2, −2, 58; z score, 4.50), bilateral dorsal prefrontal cortex (area 10) (x, y, z=−30, −58, 8; z score, 6.12; and x, y, z=50, −48, 12; z score, 5.80), right thalamus (x, y, z=−4, 20, 8; z score, 4.86), and bilateral striatum (x, y, z=−12, −6, 4; z score, 6.46; and x, y, z=8, −10, 4; z score, 6.32) (**Figure 2**) in the PSP patients compared with controls. Increases in ^{18}FDG uptake were not found in any location.

ASYMPTOMATIC MEMBERS

^{18}F-Dopa

Four of the 15 asymptomatic relatives had ^{18}F-dopa caudate and putamen uptake values that were 2.5 SDs lower than the normal mean (Figure 1). One subject was from kindred 1, and 3 subjects were from kindred 2 (**Figure 3**).

^{18}Fluorodeoxyglucose

The comparison of individual scans for each asymptomatic relative vs the control group identified 5 subjects with areas of significantly decreased regional glucose uptake.

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seconds, and the data were acquired in 3-dimensional mode as 24 time-frames over 60 minutes. In those subjects who underwent both scans, the interval between PET studies was 2 to 7 days. ^{18}F-dopa data were compared with those obtained from a group of 19 age-matched control subjects (mean±SD, 64±12.1 years) and ^{18}FDG data with those of 8 control subjects (60±14.5 years) scanned using the same camera with the same protocols.

Data Analysis

^{18}F-dopa. The analysis was performed using in-house software written in Interactive Data Language (Research System Inc, Boulder, Colo) on SUN Sparc workstations (SUN Microsystems Inc, Palo Alto, Calif). Region-of-interest (ROI) placement was defined with a standard template. We used standardized regions: a 10-mm diameter circle ROI to sample head of caudate and 10×24-mm elliptical ROI to sample dorsal putamen aligned along the long axis. These regions were placed manually by visual inspection on 3 contiguous planes encompassing the striatum. Mean counts per pixel were measured for left and right caudate and putamen in the last 14 time-frames corresponding to the period 25 to 95 minutes after injection. ^{18}F-dopa influx rate constants (K_i) were then calculated for the left and right caudate and putamen using multiple-time graphical analysis with a nonspecific occipital tissue input function.[9] Two circular regions of 32-mm diameter were placed on the occipital lobes in the same planes as those used to sample striatal regions and averaged to provide the tissue input function. Comparisons of group means were made using unpaired t tests. Individual putamen and caudate K_i values were considered abnormal if they were more than 2.5 SDs lower than the normal group means.

^{18}Fluorodeoxyglucose. The analysis was performed by applying statistical parametric mapping (SPM) to integrated images of ^{18}FDG activity spanning the last 20 minutes of the dynamic scan to identify areas of significant altered regional cerebral glucose metabolism in the PSP patients and the asymptomatic relatives compared with the control group. The validation of voxel-by-voxel statistical techniques to localize significantly altered ^{18}FDG uptake data has been recently reported.[10,11] The SPM analysis showed the same findings whether rCMRGlu (glucose regional cerebral metabolic rate) or ^{18}FDG uptake datasets were used.[10] We have chosen to use SPM and ^{18}FDG uptake data to avoid the invasive arterial cannulation required to calculate rCMRGlu.

Integral ^{18}FDG images for each subject were transformed into standard stereotactic space.[12] The template used in this study was an ^{18}FDG average image of 8 ^{18}FDG PET scans of healthy subjects normalized to the standard SPM 95 flow template.[10] The images were then smoothed using a Gaussian kernel (20×20×12-mm full width at half maximum) to remove high-frequency noise from the images. The variance in global cerebral metabolic rate for glucose across all subjects was removed using analysis of covariance; between-group comparisons were then performed with a t statistic on a voxel-by-voxel basis.[13] The first comparison aimed to identify differences in regional ^{18}FDG uptake between the 3 PSP patients and the group of 8 normal controls to establish the pattern of metabolic abnormalities in the clinically affected subjects; for this comparison, significance was accepted if voxels survived an uncorrected threshold of $P<.001$. In phantom experiments, this value of significance has been shown to be sufficiently conservative to protect against false-positive results.[14]

As most asymptomatic relatives would be expected to have normal ^{18}FDG uptake, a group analysis could mask those few subjects with significant abnormalities. We therefore compared individual scans for each asymptomatic subject against the group of 8 normal controls with the objective of finding a pattern of ^{18}FDG uptake abnormalities in those with subclinical disease similar to the affected relatives. For this comparison significance was accepted if voxels survived at a corrected threshold of $P<.01$. In this way we were able to identify 5 subjects who had at least 1 area of abnormal ^{18}FDG uptake in the same region as those of the affected relatives. We then compared these 5 subjects as a group against the group of 8 normal controls.

Four of them were also subjects with reduced striatal ^{18}F-dopa K_i values. In these 4 subjects, we found cortical and subcortical reductions of ^{18}FDG uptake similar to those found in their affected relatives. A fifth asymptomatic relative, with normal striatal ^{18}F-dopa uptake, showed a reduction of ^{18}FDG uptake in the lateral premotor cortex and dorsal prefrontal cortex (x, y, z=40, −22, 50, maximal z score, 3.17; and x, y, z=−46, −54, 8, maximal z score, 3.84, respectively).

The voxel-by-voxel analysis applied to these 5 asymptomatic members as a group showed significant decreases in ^{18}FDG uptake in bilateral lateral and medial premotor areas (x, y, z=−30, −14, 56; z score, 5.31; and x, y, z=10, −6, 52; z score, 4.24), right dorsal prefrontal cortex (x ,y, z=−46, −54, 8; z score, 5.42) and bilateral striatum (x, y, z=−6, −16, 8; z score, 4.7; and x, y, z=20, −12, 8; z score, 4.15) compared with controls (Figure 2).

Figure 3 shows the genealogical trees for kindred 1 and kindred 2. The members scanned with ^{18}FDG and/or ^{18}F-dopa and those asymptomatic subjects with abnormal scans are also indicated.

COMMENT

In our familial PSP patients, striatal ^{18}F-dopa uptake was significantly reduced bilaterally, with putamen and caudate being similarly affected. Such a uniform reduction of dopamine storage throughout the striatum has also been reported for sporadic idiopathic PSP patients[15-18] and suggests that the substantia nigra in PSP patients is globally involved. Glucose metabolism was also reduced in the striatum of our patients, in agreement with findings reported for sporadic PSP patients[19-21] and in contrast to findings for patients with PD in which striatal ^{18}FDG uptake is preserved.[19] The reduction in premotor, prefrontal, and thalamic glucose metabolism that we have found in our familial PSP members is also typical of patients with sporadic PSP,[19-22] although other areas, such as pa-

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Characteristics of the Clinically Affected Members and of the Asymptomatic Members Studied With ^{18}F-Dopa and ^{18}FDG PET*

Subject	Kindred	Sex	Age, y	Duration, y	PET Studies Performed	
					^{18}F-Dopa	^{18}FDG
Clinically affected relatives						
III.1	1	F	75	5	Yes	Yes
III.6	1	M	63	4	Yes	Yes
III.11	2	F	58	5	Yes	Yes
Asymptomatic relatives						
III.3	1	M	71		Yes	Yes
IV.1	1	M	51		Yes	No
IV.2	1	M	46		Yes	No
IV.3	1	M	41		Yes	Yes
IV.7	1	M	41		Yes	No
IV.8	1	M	40		Yes	No
			48.3 ± 11.8†		6‡	2‡
III.2	2	F	69		Yes	Yes
III.3	2	M	68		Yes	Yes
III.4	2	F	67		Yes	Yes
III.5	2	F	62		Yes	Yes
III.15	2	F	53		Yes	No
III.16	2	F	50		Yes	Yes
III.21	2	M	70		Yes	Yes
III.23	2	F	66		Yes	Yes
III.26	2	M	57		Yes	Yes
			62.4 ± 7.4†		9‡	8‡
Total			**55.4 ± 13.2†**		**15‡**	**10‡**

*^{18}FDG indicates ^{18}fluorodeoxyglucose; PET, positron emission tomography.
†Mean ± SD for kindred.
‡No. of studies performed or number. of subjects studied.

rietal cortex and cerebellum, have also been reported to be involved in this condition.[23]

Four of 15 asymptomatic relatives (27%) showed reductions in striatal ^{18}F-dopa and ^{18}FDG uptake bilaterally. These 4 subjects also had decreased ^{18}FDG uptake in a pattern similar to that of their clinically affected relatives; cortical glucose metabolism was reduced in lateral and medial premotor areas and right dorsal prefrontal cortex, while left dorsal prefrontal cortex and thalamus were spared. In addition to these 4 relatives, we identified a fifth asymptomatic subject with normal striatal ^{18}FDG and ^{18}F-dopa uptake who had reduced glucose metabolism in the premotor cortex bilaterally and the right dorsal prefrontal cortex. If we include this last subject, the percentage of asymptomatic adult relatives with abnormal PET findings increases to 33%.

Since the pattern of cerebral glucose hypometabolism and reduction of ^{18}F-dopa uptake in the clinically asymptomatic relatives is similar to that observed in the cases with established disease, we assume that these 5 among the 15 subjects at risk for PSP indeed have subclinical disease. In support of this assumption, 1 of the relatives with abnormal ^{18}F-dopa and ^{18}FDG scans developed clinical PSP 2 years after scanning at age 59 years (kindred 2, III.26). When we arbitrarily divided the asymptomatic relatives who underwent PET scanning into groups older and younger than age 50 years, we observed that none of the 4 subjects younger than 50 years had subclinical abnormalities, while the percentage of subclinical abnormalities among the 11 subjects who were aged 50 years or older rose to 45%. Since the mean age of onset of the disease in the two families is 61 years, this additional finding implies that the duration of the subclinical phase of PSP, at least in these families, is only a few years.

The reduced ^{18}FDG uptake in the frontal cortex of some asymptomatic relatives suggests that frontal cortex hypometabolism constitutes an early disease marker. In agreement with this hypothesis, a previous ^{18}FDG PET/neuropsychological study conducted in a cohort of 41 PSP patients in different stages of the disease reported that, although frontal glucose uptake decreases with disease duration, frontal hypometabolism is already present in the very early stage of the disease and precedes the onset of overt frontal lobe deficits.[22]

In 1 of our asymptomatic subjects, ^{18}FDG uptake was found only to be reduced in cortical areas with sparing of subcortical structures. In early reports of PSP, the cortex was thought to be spared,[24] and this led to the concept of a "subcortical dementia" supposedly owing to an impairment of afferent stimulating systems, maybe reticular or thalamic in origin.[24,25] Subsequent postmortem studies have consistently reported neurofibrillary tangles in frontal cortex,[26-29] suggesting that at least some of the intellectual deficits in PSP are owing to lesions in the cortex itself.[29] The finding in 1 of our asymptomatic subjects of cortical hypometabolism in a pattern similar to that of his affected relatives but without subcortical involvement supports the idea that some of the dysfunction in PSP is cortical in origin and can occur very early.

The presence of subclinical cases detected with ^{18}F-dopa and ^{18}FDG PET in asymptomatic members of PSP families suggests that the familial aggregation for this disease is greater than that ascertained on the basis of clini-

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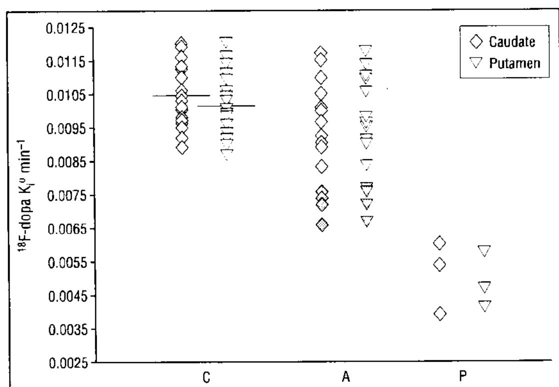

Figure 1. Caudate and putamen ^{18}F-dopa K_i values (min^{-1}) in 19 controls (C), in 15 asymptomatic members of the 2 families (A), and 3 clinically affected members (P). Symbols filled in dark gray indicate the asymptomatic relatives in that caudate and putamen ^{18}F-dopa uptake is 2.5 SDs lower than the mean caudate and putamen uptake values for the controls.

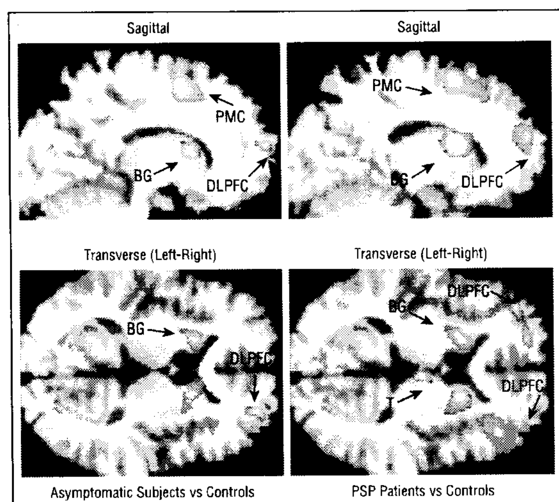

Figure 2. Areas of decreased ^{18}fluorodeoxyglucose (^{18}FDG) uptake in 3 clinically affected members compared with 8 controls (right) ($P<.001$) and in 5 asymptomatic members compared with 8 controls (left) ($P<.001$). Regions of decreased ^{18}FDG uptake have been superimposed on a normalized T1-weighted magnetic resonance imaging scan. PMC indicates premotor cortex; DPFC, dorsal prefrontal cortex; BG, basal ganglia; and T, thalamus.

cal surveys alone, indicating that PSP could have a greater hereditary component than previously realized. Factors that may explain the difficulty in recognition of familial cases of PSP on the basis of clinical findings include the late onset of the condition and the presence of occasional atypical cases given the variation in clinical phenotype. The typical syndrome is characterized by a variable combination of supranuclear ophthalmoplegia, axial dystonia, akinesia, pseudobulbar palsy, and mild dementia.[30,31] However, PSP can present with atypical clinical pictures, including a pure akinetic syndrome and pure dementia.[30,32,33] Recently, an elderly patient with pathologically confirmed PSP has been described who had a pure psychiatric syndrome without neurological signs.[34]

The prevalence of PSP in the United States[35] is reported to be 77 times lower than the prevalence of PD,[36]

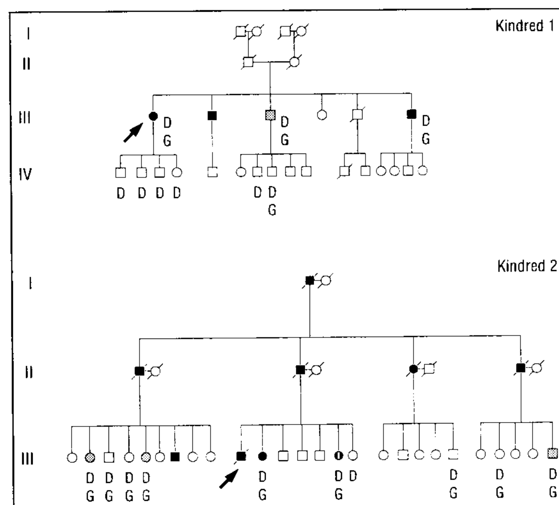

Figure 3. Genealogical trees for kindreds 1 and 2. D indicates which subjects have been scanned with ^{18}F-dopa and G with ^{18}fluorodeoxyglucose.

while, in contrast, the incidence of PSP[37] has been reported to be only 12 times lower than that of PD.[36] Although the median survival time from symptom onset is shorter in PSP[37] than in treated PD patients,[38] survival differences cannot explain the discordance in incidence and prevalence rates reported for PSP.[31] With current clinical diagnostic tools, PSP patients are diagnosed late in their disease course, and many PSP patients die with other diagnoses,[39,40] so it is likely that clinical prevalence estimates have been grossly underestimated.[31]

There have been a few previous reports of familial cases with pathologically confirmed PSP. To date, familial clustering of PSP has been reported in a total of 20 kindreds.[4] The pattern of inheritance in these reports was variable but generally suggestive of dominant transmission. Higgins et al[41] suggest that PSP can also be inherited as an autosomal recessive disorder linked to the *TAU* gene, but these data have not been confirmed. Other familial PSP clusters need to be recognized and included in a wider genetic search.

In conclusion, we have used ^{18}F-dopa and ^{18}FDG PET to assess clinically affected and asymptomatic adult members of 2 kindreds with familial PSP. Cortical and subcortical glucose and dopaminergic metabolic abnormalities with a pattern similar to that of their clinically affected relatives were found in 33% of asymptomatic adult members, suggesting that these subjects have subclinical PSP. The possibility of detecting subclinical cases and atypical phenotypes by using ^{18}F-dopa and ^{18}FDG PET could therefore improve the diagnostic recognition of PSP cases and be a valuable aid in finding a gene or genes responsible for this disease.

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REVIEW

Role of tau in Alzheimer's dementia and other neurodegenerative diseases

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Summary
Alzheimer's disease (AD) is defined histopathologically by beta-amyloid (Aβ) senile plaques and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau. The question as to which of these lesions takes precedence in AD pathology has long been an issue of debate. The amyloid cascade hypothesis, currently the predominant hypothesis, considers Aβ peptide to be responsible for the major neurodegeneration observed in AD while the cytoskeleton hypothesis states that tau hyperphosphorylation and subsequent aggregation may be central to the neurodegeneration observed in AD. This review focuses on tau mutations, phosphorylation sites, tau isoforms and the neurohistopathology of AD, and three other tauopathies to demonstrate that disease progression and neuronal loss in AD correlate also with pathological tau and not just amyloid deposition. Although tau is at the center of all these neurodegenerative diseases, there exist differences in morphology, isoforms, phosphorylation sites and mutations in each of these tauopathies. The tauopathies discussed in this review are AD, progressive supranuclear palsy, Pick's disease, and frontotemporal dementia and Parkinsonism linked to chromosome 17.

Keywords: tauopathies – progressive supranuclear palsy – Pick's disease – frontotemporal dementia – Parkinsonism

INTRODUCTION

Weingarten and colleagues first discovered a microtubule-associated protein tau (MAPT) in 1975 (Weingarten et al. 1975). Tau in the central nervous system (CNS) is found predominantly in axons of neurons, to a smaller extent in cell bodies and to an even lesser extent in dendrites (Binder et al. 1985, Papasozomenos and Binder 1987). Tau is also present in glial cells although generally under pathological conditions (Berry et al. 2001). The major function of tau in the CNS is in the stabilization of microtubules in neurons and tau might be involved in the establishment and maintenance of neuronal polarity. The C-terminus of tau binds to axonal microtubules while the N-terminus binds to neural plasma membrane components suggesting that tau functions as a linker protein between both. Besides this, tau is also involved in various signal transduction pathways where tau binds with non-receptor src family tyrosine kinases and influences neurite

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growth and the motility of microtubules in response to extracellular signals (Buee et al. 2000). However, all of these functions of tau are dependent on its ability to be phosphorylated at site-specific epitopes.

The tau gene located on human chromosome 17q21 in the human genome, contains 16 exons with the major tau isoform being encoded by 11 exons (Goedert et al. 1989, Spillantini and Goedert 1998). Alternative pre-mRNA splicing of exons 2, 3 and 10 in the single tau gene results in the formation of six different isoforms in the adult human brain (Buee et al. 2000). These isoforms ranging from 352-441 amino acids are responsible for the modulation of tau function and are characterized by the presence of three (3R tau) or four (4R tau) tandem repeats of 31-32 amino acids located in the carboxy terminal end which is also the microtubule binding domain of tau (Goedert et al. 1989). These tandem repeats of 3R and 4R are encoded by exons 9-12 (exon 10 inclusion results in the generation of 4R tau while its exclusion generates 3R tau). In the adult human brain the ratio of 3R tau to 4R tau is approximately 1 and this balance is disrupted in the case of tau mutations associated with exon 10 (Goedert and Jakes 2005, D'Souza and Schellenberg 2005). In the N-terminal region there is a presence/absence of a 29 or 58 amino acid insert (exon 2 alone or exons 2 and 3 together), respectively. This N-terminal insert is the major factor responsible for variability in the six isoforms (Buee et al. 2000). In the foetal human brain, exons 2, 3 and 10 are excluded and a single isoform is produced comprised of 316 amino acids (D'Souza and Schellenberg 2005). Foetal tau in the CNS is more highly phosphorylated than tau in the adult brain.

Various kinases and phosphatases are involved in the regulation of tau phosphorylation that occurs at a number of serine, threonine and proline residues (Butler and Shelanski 1986, Ferrer et al. 2005). Tau hyperphosphorylation is at the crux of most tauopathies since hyperphosphorylation dissociates tau from microtubules, destabilizes them and forms paired helical filaments (PHF) *in vitro* (Lindwall and Cole 1984, Alonso et al. 1994). Tau phosphorylation is regulated by an exquisite equilibrium between kinase and phosphatase activities. An imbalance of these two enzymatic processes can result in abnormal hyperphosphorylation of tau and the generation of PHF. Mutations in the tau gene and tau hyperphosphorylation have been observed in many neurodegenerative diseases as well as in senescent brains. Neurodegenerative diseases of note include Pick's disease, AD, frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), corticobasal degeneration, Niemann Pick's disease, etc. These diseases are all referred to as tauopathies since they all share a common pathology which is aggregated tau. This review focuses on four disorders, since each differs substantially in their tau pathology, especially with respect to AD.

ALZHEIMER'S DISEASE (AD)

AD is named after Dr. Alois Alzheimer, a German physician who observed changes in the brain tissue of a woman in 1906 (Moller and Graber 1998). To date, the cause and progression of sporadic AD have not been fully elucidated. AD is neuropathologically characterized by the presence of extracellular amyloid deposits and intracellular NFTs composed of hyperphosphorylated tau. NFTs are preferentially observed in hippocampal cells and the entorhinal cortex. In addition, many cortical and sub-cortical areas such as the amygdala and dorsal raphe nucleus are affected also by NFT formation. Symptoms of the disease include memory loss followed by aphasia, agnosia, apraxia and behavioral disturbances. The disease generally affects people over 65 years of age.

The autosomal-dominant inherited forms of early-onset Alzheimer's disease are caused by mutations in the genes encoding the amyloid precursor protein (APP), presenilin 1 (PS-1) on chromosome 14, and presenilin 2 (PS-2) on chromosome 1 (Buee et al. 2000). A polymorphism in the apolipoprotein E gene (the *E4* allele) is also a genetic risk factor associated with late-onset AD (Wilhelmus et al. 2005). Studies supporting the amyloid cascade hypothesis suggest that amyloid is upstream of tau and could be a significant factor in hyperphosphorylating tau, resulting in the formation of neurofibrillary tangles followed by neurodegeneration (Hardy and Selkoe 2002). This theory is supported by various transgenic mouse models of AD that have been studied in the recent past to monitor effects of amyloid beta peptide (Aβ and tau on cognition and memory deficits, in an attempt to mimic human AD neuropathology of plaques and tangles (Gotz et al. 2001). In the triple transgenic mice with *APP*, *PS-1* and *P301L* mutations, intracerebral injection with anti-Aβ antibodies decreased tau pathology in younger mice further supporting the claim that Aβ could be upstream of tau (Oddo et al. 2004). However, other studies in transgenic mice have demonstrated *MAPT* mutations in combination with *APP* mutations show more neurodegeneration as opposed to *APP* mutations alone (Lewis et al. 2001).

Immunoblotting of AD tau proteins isolated from aggregated PHF's reveals the presence of a major tau triplet (tau 55, 64 and 69 kd) and an additional minor 72-74 kd component in the case of AD thereby differentiating it from the other

tauopathies (Mailliot et al. 2000). Also via immunoassay techniques, pathologic tau with distinctive morphology and specificity to AD can be detected. Tau hyperphosphorylation in AD results in the reduced ability of tau to bind to microtubules. Hyperphosphorylation of tau in AD generates differences that can be visualized by phosphorylation-dependent antibodies that include AT100, AP422, 988, TG3 and PHF-27 (Augustinack et al. 2002a).

In AD, tau is hyperphosphorylated at 30 specific amino acid sequences throughout its 441 amino acids (Gong et al. 2005). *In vitro* studies suggest that increases in Aβ production may potentiate tau phosphorylation by activation of kinases such as glycogen synthase kinase-3 (GSK-3) (Alvarez et al. 1999). Phosphorylated tau protein at T231 is seen in the postmortem brain tissue of patients with AD and can be detected in cerebrospinal fluid (Buerger et al. 2002). Additional studies indicate that increased activation of the stress related kinases JNK and p38 occurs very early in the disease and might be involved in the intraneuronal protein phosphorylation/ dephosphorylation imbalance that leads to neurofibrillary degeneration in AD (Reynolds et al. 2000, Pei et al. 2001). It is noteworthy that p35/Cdk5 kinase complex can phosphorylate human tau at seven sites S195, S202, T205, T231, S235, S396 and S404 (Paudel et al. 1993). Also, *in vitro* studies show increased levels of p25, an activator of cdk5 that phosphorylates tau at S199, S202, and T205 (Augustinack et al. 2002b). And, immunoblotting studies using anti-pS208 and anti-pS210 show that tau-tubulin kinase phosphorylates S208 and S210 in PHF-tau (Tomizawa et al. 2001).

The phosphorylation sites and immunohistochemistry of tau in AD differentiate it from the other tauopathies. In addition, there are no known mutations in tau that are associated with AD as opposed to the other tauopathies (Gotz et al. 2006). Although Gerstmann-Straussler-Scheinker disease (GSS), a prion disease has amyloid plaques as a result of pathological prions they differ in tau pathology from AD morphologically and also with respect to differences in tau isoforms and by immunohistochemistry. In contrast to AD, GSS shows low levels of the major tau triplet (tau 55, 64 and 69 kd) (Mailliot et al. 2000).

PROGRESSIVE SUPRANUCLEAR PALSY (PSP)

PSP, the second most frequent cause of degenerative Parkinsonism was identified by Clifford Richardson, Steele and Olszewski in 1964 (Steele et al. 1964). Neuropathologically, PSP is characterized by abundant neurofibrillary tangles that are primarily localized to subcortical regions and are found in both neurons and glia. Clinical phenotypes of PSP are similar to Parkinson's disease and include unsteady gait, stiff movements and mild dementia due to a selective loss of caudal intralaminar nuclei that result in the loss of dopaminergic neurons in the substantia nigra (Henderson et al. 2000). Other symptoms include blurred vision, downward gaze palsy, followed by depression, sleeplessness, memory loss, dysphagia and dysarthria. Men are more prone to this disease than women and the age of onset varies from the early forties to late eighties.

The etiology of this disease is not fully understood but data implicate polymorphisms in the tau gene as a risk factor. Conrad et al. (1997) first identified a dinucleotide polymorphism involving a TG repeat in intron 9. The TG repeat of 11 dinucleotides is termed the *A0* allele, while the *A1*, *A2*, *A3* and *A4* alleles represent 12, 13, 14 and 15 dinucleotide repeats, respectively. Subsequent studies have shown that this polymorphism is inherited as part of two extended haplotypes (H1 and H2) that cover the entire tau gene (Baker et al. 1999). The *A0*, *A1* and *A2* alleles are present in haplotype H1 while *A3* and *A4* are part of haplotype H2. However, these polymorphisms are not specific to PSP because *A0* allele and H1 haplotype are also implicated in corticobasal degeneration (Houlden et al. 2001). Mutations in the tau gene associated with neuropathology typical of PSP include a silent mutation S305S in exon 10, a R5L in exon 1, and G303V mutation in exon 10 (Stanford et al. 2000, Poorkaj et al. 2002, Ros et al. 2005a) (Table 1). All of the above mutations result in the predominant tau isoform being 4R tau. The deletion of asparagine at codon 296 (del296) of the tau gene causes atypical PSP in patients homozygous for this mutation while the heterozygous mutation phenotypically resembles Parkinson's disease (Grover et al. 2002). The S352L homozygous tau gene mutation found in two English siblings showed tau neuropathology different from FTDP-17 cases and functional studies showed reduced microtubule assembly and increased aggregation of tau. Both siblings carried the H1/H1 haplotype associated with PSP (Nicholl et al. 2003). In addition to mutations in the tau gene, studies have also shown linkage of PSP to a new locus *1q31.1* in chromosome 1 (Ros et al. 2005b).

Pathological and biochemical studies in PSP brains have shown a predominance of hyperphosphorylated aggregated 4R tau isoforms (Litvan and Hutton 1998). Tau pathology in the postmortem PSP brain may include neuropil threads, neuritic plaques, tufted astrocytes and glial inclusions, as well as microglia and globose tangles (Morris et al. 2002). Ultrastructural analyses have revealed differences in AD and PSP pathology. Tau

morphology in AD is PHFs whereas the tau filaments are straight in PSP (Spillantini and Goedert 1998, Morris et al. 2002). In addition, electrophoretic profiles of aggregated tau proteins in PSP differ from that of AD. In AD all six isoforms of tau are phosphorylated and the aggregated tau, as detected by immunoblotting appears as a major tau triplet (tau 55, 64 and 69 kd) while in PSP only the 4R isoforms are phosphorylated and appear as a major tau doublet (tau 55 and 64 kd) (Mailliot et al. 2000).

The kinases that may cause hyperphosphorylation of tau in PSP are phospho-p38 MAPK, and the stress kinases SAPK/JNK-P which are upregulated in neurons, astrocytes and oligodendroglia displaying aggregated tau (Ferrer et al. 2001). Another kinase that is implicated in abnormal phosphorylation of tau in PSP is casein kinase 1 delta (Schwab et al. 2000).

PICK'S DISEASE

Arnold Pick first described Pick's disease in 1892 (Karenberg 2001). Pick's disease differs from AD in several aspects. It is marked by rounded microscopic structures called Pick's bodies rather than the plaques and tangles of AD. Pick's disease is characterized by frontotemporal lobar atrophy, gliosis and ballooned neurons. The first symptoms associated with this disease are personality changes, poor social judgement, difficulty with language and poor attention span. The onset is usually at 40 years of age and is less common after 60 years of age.

Pick's disease is also referred to as one of the fronto-temporal dementias (FTDs) caused by mutations in the tau gene in exons 9, 12, 11 and 13 (Murrell et al. 1999, Yen et al. 1999, Rizzini et al. 2000, Pickering-Brown et al. 2000, Neumann et al. 2001, Rosso et al. 2002, Hogg et al. 2003, Bronner et al. 2005) (Table 1). All mutations associated with Pick's disease and identified in table 1 show a reduced microtubule assembly of tau. Functional analysis of tau with G389R and K257T mutations have demonstrated increased susceptibility of tau to calpain I digestion (a feature probably related to the formation of a Pick's disease type of histology) in addition to reduced microtubule assembly of tau (Pickering-Brown et al. 2000). Besides mutations in the tau gene, a novel mutation G183V in the presenilin-1 (PS-1) gene affects the splice signal at the junction of the sixth exon and intron resulting in clinical manifestations similar to Pick-type tauopathy, in the absence of extracellular beta-amyloid deposits (Dermaut et al. 2004). Subsequent studies showed that a mutation in PS-1, M146L which accounts for most cases of familial AD can also cause Pick's disease (Halliday et al. 2005). PS-1 mutations are known to enhance gamma-secretase activity resulting in an increase in Aβ42 (Haass and De Strooper 1999). However, PS-1 mutations in addition to increasing Aβ42 also increase the production of total tau proteins (Shepherd et al. 2004). The mechanism of action of M146L mutation is hypothesized to predispose the person to Pick's disease, or AD, or both, by affecting multiple intracellular pathways involved with tau phosphorylation, resulting in 3R isoforms and amyloid metabolism (Halliday et al. 2005).

Histopathologically, Pick's bodies are intraneuronal inclusions of tau, and only phosphorylated 3R-tau isoforms aggregate into filaments. They are characterized as a major tau doublet (tau 55 and 64 kd) (Mailliot et al. 2000). However, studies have also revealed 4R aggregated tau isoforms in patients with sporadic Pick's disease, although the 3R isoforms are more predominant (Zhukareva et al. 2002). Interestingly, analysis of dephosphorylated tau from the brain of a patient with the G389R mutation revealed a prominent tau band with 4R isoforms and no amino terminal inserts (Murrell et al. 1999). Ultrastructurally, Pick's bodies consist of both random coiled and straight filaments. Further, aggregated tau proteins in Pick's disease are not detected by the monoclonal antibody 12E8 that binds to phosphorylated Ser262/356 residues, as is the case with other neurodegenerative diseases. This lack of 12E8 immunoreactivity suggests either a kinase inhibition in neurons that degenerate in Pick's disease or an absence of the related kinases (Probst et al. 1996). Furthermore, the active stress kinase p38 has been shown to enhance abnormal tau phosphorylation in Pick's disease (Puig et al. 2004). The other kinases implicated in Pick's disease are similar to those involved in PSP.

FRONTOTEMPORAL DEMENTIA AND PARKINSONISM LINKED TO CHROMOSOME 17 (FTDP-17)

The name frontotemporal dementia was first proposed by the Lund and Manchester group in 1994 (Mori 2004). Wilhelmsen and colleagues renamed FTD as FTDP-17 after observing clinical and pathological features of frontotemporal dementia in patients with genetic defects in chromosome 17q21-22 (Buee et al. 2000). FTDP-17 is characterized by behavioural, cognitive and motor disturbances that are caused by lesions in the frontotemporal regions of the brain. The pattern of inheritance in FTDP-17 is autosomal dominant with an early age of onset between 45-65 years of age. Pathological changes in the brain include frontotemporal atrophy with neuronal loss, gray and white matter gliosis and superficial cortical spongiform.

Table 1. **Tau mutations on chromosome 17**

Mutations	Exon location	Pathogenic effects of Tau mutations	Disease Pathology	Literature cited
R5H	1	Increased aggregation of tau protein, reduced MT assembly and all six isoforms affected	AD-like	Hayashi et al. 2002, D'Souza and Schellenberg 2005
R5L	1	Increased aggregation of tau protein, reduced MT assembly and all six tau isoforms affected	PSP	Poorkaj et al. 2002
K257T	9	Increased aggregation of tau protein, reduced MT assembly and all six tau isoforms affected	Pick's disease	Rizzini et al. 2000
I260V	9	Increased aggregation of tau protein, reduced MT assembly and all six tau isoforms affected	FTDP-17	Brandt et al. 2005
L266V	9	Increased aggregation of tau protein, reduced MT binding and all six tau isoforms affected, increases splicing of exon 10	Pick's disease	Hogg et al. 2003, D'Souza and Schellenberg 2005
G272V	9	Increased aggregation of tau protein, reduced MT binding, all six tau isoforms affected	Pick's disease, FTDP-17	Bronner et al. 2005, Hutton et al. 1998, Spillantini and Goedert 1998
N279K	10	Increase splicing of exon 10 changing 4R:3R ratio	FTDP-17	Sergeant et al. 2005, D'Souza and Schellenberg 2005
delK280	10	Increased aggregation of tau protein, decreases splicing of exon 10, reduced MT assembly	FTDP-17	Brandt et al. 2005
L284L	10	Increase splicing of exon 10 changing 4R:3R ratio	AD-like	D'Souza and Schellenberg 2005
Delta N296	10	Reduces microtubule assembly of tau and increases tau aggregation	PSP & FTDP-17	Grover et al. 2002, Yoshida et al. 2002
N296H	10	Increases splicing of exon 10, increases tau aggregation and reduces tau promoted tubulin polymerization	FTDP-17	Grover et al. 2002
N296N	10	Increases splicing of exon 10 thereby increasing ratio of 4R/3R tau	FTD	Grover et al. 2002
P301L	10	Increased aggregation of tau protein, does not affect splicing of exon 10, reduced MT binding, 4R tau isoforms	FTDP-17	Hutton et al. 1998
P301S	10	Increased aggregation of tau protein, does not affect splicing of exon 10, reduced MT binding	FTDP-17	Sergeant et al. 2005
G303V	10	Increases splicing of exon 10 increasing 4R/3R ratio	PSP	Ros et al. 2005a
S305N	10	Increase microtubule assembly, increases splicing of exon 10 increasing 4R/3R ratio	Pick's disease	Sergeant et al. 2005, D'Souza and Schellenberg 2005
S305S	10	Increase splicing of exon 10 increasing 4R/3R ratio	PSP	Stanford et al. 2000
L315R	11	Reduced MT assembly	Pick's/ FTDP-17	Brandt et al. 2005
K317M	11		FTDP-17	Zarranz et al. 2005
S320F	11	Reduced MT assembly	Pick's disease pathology	Rosso et al. 2002, Brandt et al. 2005
Q336R	12	Increase microtubule assembly, Increased aggregation of tau protein	Pick's disease pathology	Pickering-Brown et al. 2004
V337M	12	Increased aggregation of tau protein, reduced MT binding	AD-like	Poorkaj et al. 1998

Continued Table 1

E342V	12	Increased exon 10 splicing, no effect on MT assembly	FTDP-17/ Pick's	Lippa et al. 2000, Brandt et al. 2005
S352L	12	Reduced MT assembly and increased aggregation of tau protein	PSP	Nicholl et al. 2003
K369I	12	Reduced MT assembly	Pick's disease pathology	Neumann et al. 2001
G389R	13	Reduced MT assembly	Pick's / FTD.	Murrell et al. 1999
R406W	13	Increased aggregation of tau protein, reduced MT assembly	PSP	Hutton et al. 1998
T427M	13		FTDP-17	Giaccone et al. 2005

Most of the known mutations causing FTDP-17 occur in the C-terminal end of tau and affect mainly exons 9-12 that encode the microtubule binding repeats. The different tau mutations give rise to different pathological characteristics depending on their location. Missense mutations outside exon 10 result in the formation of straight neuronal filaments in all six isoforms. These filaments resemble the PHFs observed in AD. In contrast, missense or splice mutations that directly affect exon 10 result in both neuronal and glial tau pathology with filaments consisting predominantly of 4R tau isoforms (van Slegtenhorst et al. 2000).

Tau mutations causing FTDP-17 either alter the ratio of 4R:3R tau through faulty splicing of exon 10 or directly impair the binding of tau to microtubules and its subsequent aggregation properties. FTDP-17 mutations affecting exon 10 are presented in Table 1. The intronic mutations in the 5' splice site of exon10 are E10+3, E10+11, E10+12, E10+13, E10+14, E10+16, E10 +33, E10+19, E10 +29 (Hutton et al. 1998, van Slegtenhorst et al. 2000, Mack et al. 2001, Morris et al. 2003, Sergeant et al. 2005). Exon 10 and intronic mutations following exon 10 affect the cellular functioning and biochemical expression of tau by changing the normal 1:1 ratio of 3R:4R isoforms. Most of the intronic mutations increase splicing of exon 10 except E10+19 and E10+29 (which decrease the splicing of exon 10) and result in the predominant tau isoform being 4R. Mutations affecting exon 10 cause ribbon twisted filaments of mostly 4R tau that do not bind to microtubules. Mutations outside of exon 10 result in PHF and straight filaments generally affecting all 6 isoforms that do not bind to microtubules (Sergeant et al. 2005). Most of these mutations alter the ability of tau to interact with microtubules thereby increasing the likelihood that tau will assemble into filaments (Brandt et al. 2005). However, other mutations like delN296, N296N and N296H reduce the ability of tau to promote assembly of microtubes, without having a significant effect on tau filament formation (Yoshida et al. 2002).

Other mutations involved with FTDP-17 include a R5H mutation in exon 1 which reduces microtubule assembly and promotes formation of fibrils *in vitro*, a T427M mutation in exon 13 identified in an Italian patient with a family history of FTD, and the E342V mutation in exon 12 which preferentially increases 4R tau (Hayashi et al. 2002, Giaccone et al. 2005, Lippa et al. 2000 respectively). The Q336R mutation in exon 12 of the tau gene increases microtubule assembly of tau *in vitro* while an adjacent mutation V337M decreases microtubule assembly of tau (Pickering-Brown et al. 2004). The tau pathology associated with these two different mutations although adjacent to each other is also different. The former mutation results in Pick-type tau histology while the latter has NFTs similar to AD, like tau pathology (Poorkaj et al. 1998, Pickering-Brown et al. 2004). Another mutation implicated in FTDP-17 is the K317M mutation located in exon 11 of the *MAPT* gene. Biochemical analysis of brain homogenates revealed two bands of phospho-tau at 64 and 68 kd (Zarranz et al. 2005). However, there have been many cases of familial FTDP-17 with no detectable mutations in the tau gene. Recent studies have shown that these cases of familial FTDP-17 are caused by mutations in the progranulin gene situated just next to the tau gene (Baker et al. 2006, Cruts et al. 2006).

Studies on FTDP-17 mutations expressed in differentiated neuronal cells reveal decreased phosphorylation of pathologically relevant S202/T205 sites, but phosphorylation at the S396/S404 site is moderately decreased for all mutant isoforms (Furukawa et al. 2003). Other studies suggest that mutations in tau that decrease its microtubule-binding capacity augment calcium influx by depolymerizing microtubules and activating adenyl cyclase and protein kinase A (Buee-Scherrer et al. 2002). Transgenic mouse models with the different mutations of tau have been developed to study the effects of tau pathology on cognition and memory. Some of these mouse models have been successful in mimicking the molecular and cellular features of the human

disease in terms of hyperphosphorylation and filament formation (Goedert and Jakes 2005).

DISCUSSION

Tau, a microtubule-associated protein, is abnormally hyperphosphorylated in senescent tissue and in a number of neurodegenerative diseases collectively referred to as tauopathies. There is no known cure to date for each of the tauopathies discussed in this review. All available therapies provide only symptomatic relief. The key challenge facing scientists in the area of neurodegenerative diseases is the need to develop effective therapeutic agents that could lead to a cure for these diseases.

Tau is a substrate for various protein kinases *in vitro*, namely, the Ca++/Calmodulin dependent protein kinase II, Casein kinase II, GSK3, the MAPK, also known as ERK, cdk5 and the microtubule affinity regulating kinase (MARK). The stress activated protein kinases SAPK3/p38gamma and SAPK4/p38delta also cause abnormal hyperphosphorylation of tau (Buee-Scherrer et al. 2002). Each kinase phosphorylates different residues that are proline or non-proline specific. GSK3 phosphorylates S199 but not detectably S202 or T205. The MAPKs may not be strictly proline specific. p38 phosphorylates the nonproline sites S185, T245, S305, and S356. The MAPKs and GSK3 are important tau kinases that may be involved in the pathogenic hyperphosphorylation of tau in AD (Paudel et al. 1993). Phosphorylated tau is dephosphorylated by numerous protein phosphatases *in vitro*. Phosphatase 1, phosphatase 2A and the Ca++/calmodulin-dependent phosphatase, phosphatase 2B all have been shown to dephosphorylate tau (Gong et al. 1994a, Gong et al. 1994b). Dephosphorylation of tau by these phosphatases increases the ability of tau to bind to microtubules and also promotes microtubule assembly.

In vitro models used to verify these results are PC12 cells and SH-SY5Y neuroblastoma cells.

Table 2. **Summary of the tauopathies**

Disease	Tau repeats	Age of onset (years)	Literature cited
Pick's	3 repeat	40-60	Pickering-Brown et al. 2004, Mailliot et al. 2000
PSP	4 repeat	Early 40 - late 80	Mailliot et al. 2000, Morris et al. 2003
AD	3 repeat + 4 repeat	Over 65	Mailliot et al. 2000, Gotz et al. 2006
FTDP-17	3 repeat + 4 repeat	45 -65	Mailliot et al. 2000, Pickering-Brown et al. 2004

However, it is also important to mention at this stage that tau hyperphosphorylation alone is not the predisposing factor for all these tauopathies. Other factors like oxidative stress, enzyme regulation, cellular vulnerability and genetic mutations also play a major role in the pathogenicity of tauopathies. Although mutations in PS-1 account for the majority of familial cases of AD and cause an increased production of Aβ42, little is known about the role of tau in PS-1 AD. There is a 6-fold increase in tau-2-positive plaques in PS-1 cases thereby suggesting that PS-1 mutations increase tau hyperphosphorylation perhaps by increasing Aβ, which in turn activates GSK-3 (Shepherd et al. 2004). This theory supports the amyloid cascade hypothesis of amyloid being upstream of tau in AD.

Various transgenic mice have been developed to study the effects of tau mutations and the various kinases and phosphatases on tau, and to monitor the neuropathology *in vivo*. There has been some success in the latter with the development of transgenic mice expressing wild type tau and mutant tau P301L (Lewis et al. 2000). Other transgenic mouse models have included glial pathology in addition to neuronal pathology (Higuchi et al. 2002). Although transgenic mice with the mutant V337M mutation are said to show behavioral and pathological features similar to AD (Tanemura et al. 2002), they still do not completely simulate or mimic the conditions in the human brain. The triple transgenic mouse (APP, PS-1 and tau) model shows the closest resemblance of AD pathology in humans with respect to plaques and

tangles (Oddo et al. 2003). Researchers have also tried to mimic human AD pathology in monkeys with a view to finding suitable therapeutic agents to treat the disease. However, the drawback of this model of AD is the extended time period (around 10 years) necessary for Aβ deposition in these primates (Ridley et al. 2006). To further investigate the hypothesis that NFTs are responsible for brain dysfunction and neurodegeneration, transgenic mice expressing mutant tau that could be suppressed with doxycycline were created (Santacruz et al. 2005). These mice showed improved memory function after suppression of human mutant tau but did not prevent the accumulation of NFTs. Also, MAPT mutations along with APP mutations cause more neurodegeneration in AD mouse models than APP mutations alone (Lewis et al 2001) and studies from human tissue with AD show that NFTs correlate better with impairment than amyloid (Arriagada et al 1992). Altogether, these results suggest that abnormal tau correlates more with the pathogenesis of AD than amyloid. And, although transgenic mice have allowed considerable progress in our understanding, they still present limitations for modeling human AD. An overview of the different isoforms of tau in various tauopathies including age of onset is presented in Table 2. Both amyloid and tau hypotheses have their strengths and drawbacks and continued efforts should be made in both areas towards finding a cure for AD and other tauopathies.

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Clinical genetics of familial progressive supranuclear palsy

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Summary

Recent studies have shown that progressive supranuclear palsy (PSP) could be inherited, but the pattern of inheritance and the spectrum of the clinical findings in relatives are unknown. We here report 12 pedigrees, confirmed by pathology in four probands, with familial PSP. Pathological diagnosis was confirmed according to recently reported internationally agreed criteria. The spectrum of the clinical phenotypes in these families was variable including 34 typical cases of PSP (12 probands plus 22 secondary cases), three patients with postural tremor, three with dementia, one with parkinsonism, two with tremor, dystonia, gaze palsy and tics, and one with gait disturbance. The presence of affected members in at least two generations in eight of the families and the absence of consanguinity suggests autosomal dominant transmission with incomplete penetrance. We conclude that hereditary PSP is more frequent than previously thought and that the scarcity of familial cases may be related to a lack of recognition of the variable phenotypic expression of the disease.

Keywords: progressive supranuclear palsy; Steele–Richardson–Olzsewski syndrome; genetics; akinetic rigid syndrome; dementia

Abbreviation: PSP = progressive supranuclear palsy

Introduction

Progressive supranuclear palsy (PSP) is a neurodegenerative disease clinically characterized by a variable combination of akinesia, supranuclear gaze palsy, rigidity, axial dystonia, gait disturbance and frontolimbic dementia. Pathological abnormalities include neuronal loss, gliosis and the presence of neurofibrillary tangles and neuropil threads, mainly in basal ganglia, diencephalon, brainstem and frontal and temporal lobes (Steele *et al.*, 1964). Atypical cases with characteristic pathological findings but an incomplete clinical syndrome have been previously described (Hauw *et al.*, 1994; Collins *et al.*, 1995; Daniel *et al.*, 1995; Litvan *et al.*, 1996*a*; Verny *et al.*, 1996).

The cause is unknown but toxic and infectious aetiologies have been considered, based upon the pathological similarities with post-encephalitic parkinsonism, metal poisoning and with the Parkinson–dementia complex of Guam (Jellinger, 1971; Steele, 1972, 1975; Jankovic, 1984; Kristensen, 1985; Jendroska *et al.*, 1994; Lilienfeld *et al.*,1994). Because of the coexistence of cerebrovascular disease in some cases a vascular mechanism has also been postulated (Dubinsky and Jancovic, 1987; Winikates and Jankovic, 1994). PSP is still considered a sporadic disorder, despite a small number of recent reports suggesting familial clustering (David *et al.*, 1968; Mata *et al.*, 1983; Ohara *et al.*, 1992; Brown *et al.*,

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1993; Gazely and Maguire, 1994; Tetrud *et al.*, 1994; de Yébenes *et al.*, 1995; Golbe *et al.*, 1995; Lanotte *et al.*,1996; Tetrud *et al.*, 1996). In view of the few families reported it is not possible to decide whether familial and sporadic PSP are the same disease.

In this study we investigated our cases of PSP in order to describe familial aggregation, clinical phenotypes and pattern of inheritance.

Methods

A retrospective study of all patients with familial PSP seen by or referred to one of us (J.G.Y.) during the period 1991–97 was carried out. The index cases were seen at the Movement Disorders Clinic, Department of Neurology, Fundación Jiménez Díaz (five cases), the Institute of Neurology, Queen Square, London (two cases), Hospital General de Segovia (one case), Hôpital Neurologique, Lyon (one case), Columbia University, New York (two cases) and Kingston General Hospital, Ontario (one case). Review of medical records, professional or domestic videos, photographs, samples of hand writing, telephone calls and visits to the homes of the patients and relatives were undertaken by members of the research team when needed in order to evaluate secondary cases.

The diagnosis of PSP in a proband required either (i) pathology proven diagnosis according to international criteria (Hauw *et al.*, 1994; Litvan *et al.*, 1996*a*), (ii) the presence of the international clinical research criteria for the diagnosis of PSP (Litvan *et al.*, 1996*b*) or (iii) in some cases, analysed retrospectively, with insufficient details in the available history to fulfil the international clinical research criteria, the diagnosis of PSP was accepted if the patients had at least five out of the seven most common clinical symptoms of the disease (bradykinesia, gait disturbance, supranuclear gaze palsy, dysphagia, dysarthria, axial dystonia or disabling mental changes with frontosubcortical characteristics) as described in a recent clinicopathological series (Daniel *et al.*, 1995). The presence of these signs was determined clinically. Supranuclear gaze palsy was defined by saccades smaller than 15° in the vertical or horizontal plane. Whenever possible, in patients seen at Fundación Jiménez Díaz, supranuclear gaze palsy was confirmed by oculo-nystamographic analysis. Abnormal findings were defined by latency, velocity or accuracy of saccadic movements more than 2 SD away from the mean for that age group.

When the available information was insufficient to make a reliable diagnosis of PSP, but it was suggested by the relatives describing the phenotype as 'similar' or 'the same' as the proband, the individual was diagnosed as 'likely PSP'. We respected the initial diagnosis of other neurological disorders including parkinsonism, dementia, etc. when there was not additional clinical information that allowed for reclassification.

We obtained information on all available or deceased first and second degree relatives (parents, brothers, sisters, uncles, cousins) of the probands when possible. Children of probands were excluded since they were too young to clinically express PSP.

Results

Description of the families

A brief description of the pedigrees is presented in Table 1 and Fig. 1 (relationship, age of onset, years of evolution, medical history and clinical diagnosis). Detailed clinical phenotypes and response to L-dopa therapy are described in Table 2.

Family 1

Proband, individual 1.III.12. This was a 57-year-old female who presented progressive difficulty in doing up buttons and turning in bed. She had a long history of smoking and hypercholesterolaemia. At the age of 60 her neurological examination revealed moderate axial and limb rigidity, and dystonia in the left arm. She was diagnosed as having Parkinson's disease and treated with L-dopa, which improved her symptoms but induced akathisia and orolingual dyskinesias. She was then seen by one of us (J.G.Y.) at age 65. She complained of slowness, gait disturbance, speech problems with hypophonia and progressive dysphagia. A bruit was heard over the left carotid artery. Mental status and cranial nerves were normal with the exception of limitation of downgaze. She had dystonic posturing of the neck (anterocollis) and dystonic up-going toes. She showed severe, generalized akinesia and postural instability with a tendency to fall backwards. MRI of the brain was normal. She became unresponsive to L-dopa and pergolide and died at the age of 67.

The macroscopic examination of the brain revealed a pale substantia nigra. Light microscopy examination showed neuronal loss, gliosis and a high density of neurofibrillary tangles in the globus pallidus, putamen, subthalamic nucleus, substantia nigra and the inferior olivary nucleus. There were a moderate number of tangles in the neocortex (predominantly in anterior frontal and parietal regions), hippocampus, amygdala, nucleus basalis of Meynert and locus coeruleus. Tangles were also identified in both colliculi, peri-aqueductal region, red nucleus, dentate nucleus and oculomotor complex.

Individual 1.III.3. This was the first cousin of the proband, with a history of diabetes. His neurological disorder began with a progressive slowness of the left arm and leg when he was 68 years old. He was thought to have Parkinson's disease and received treatment with L-dopa with improvement of his symptoms. At the age of 73 he was evaluated by one of us (J.G.Y.) and the neurological examination revealed slowness, clumsiness, severe dysphagia and diplopia. His physical examination revealed a bruit over the left internal carotid artery. Formal neuropsychological testing revealed bradyphrenia and abnormalities of executive memory and

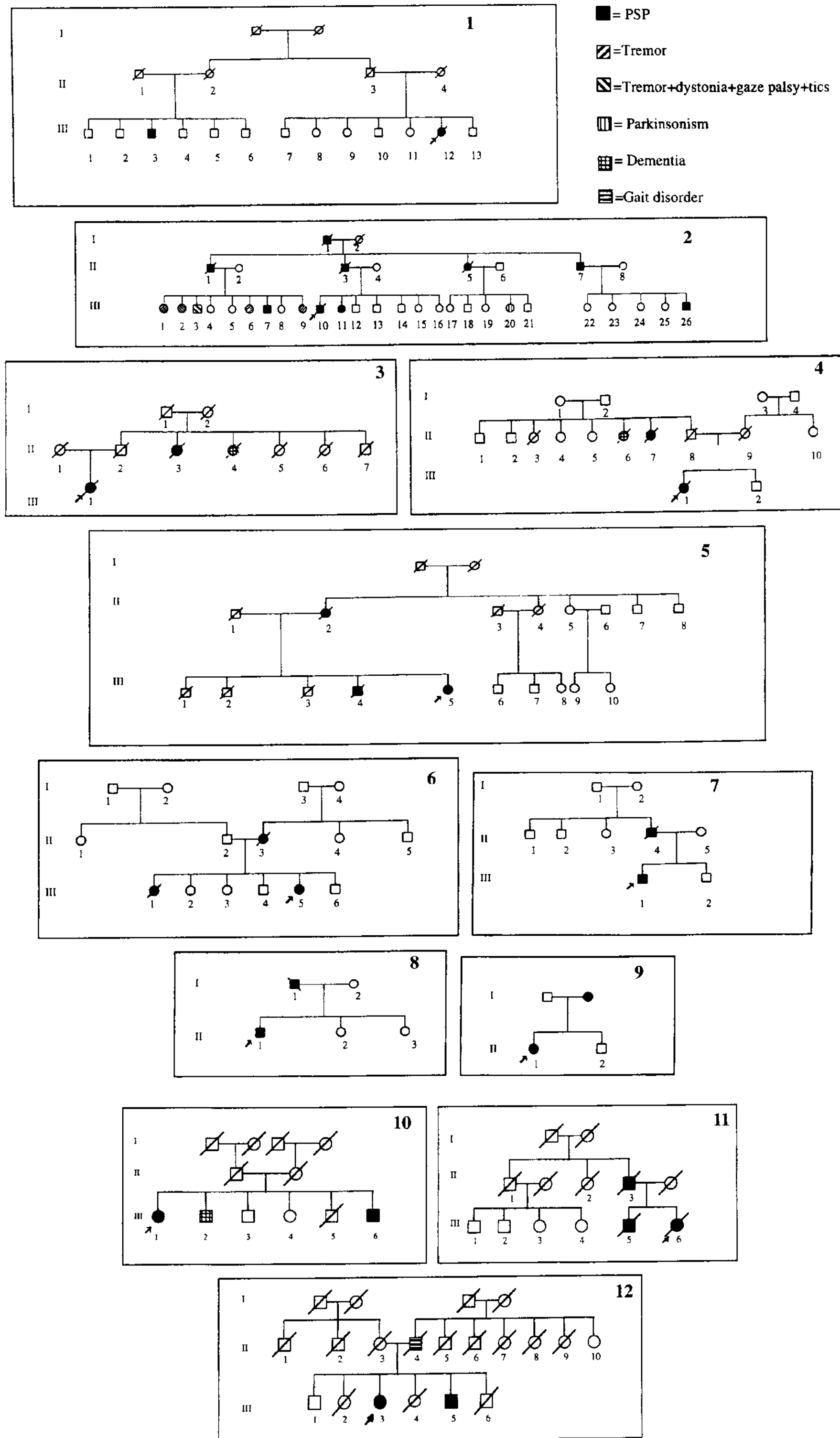

Fig. 1 Familial trees of families 1–12. Arrows point to probands.

Table 1 *Epidemiological data*

Family	Number		Sex	Age at onset (years)	Actual age (years)	Age at death	Actual diagnosis
1	III.12	Proband*	F	57	–	67	PSP
	III.3	Cousin	M	68	73	–	PSP
2	III.10	Proband*	M	53	–	59	PSP
	I.1	Grandfather	M	NA	–	NA	Likely PSP
	II.1	Uncle	M	NA	–	81	PSP
	II.3	Father	M	62	–	71	Likely PSP
	II.5	Aunt	F	70	–	84	PSP
	II.7	Uncle	M	NA	–	77	PSP
	III.1	Cousin	F	NA	73	–	Tremor
	III.2	Cousin	F	NA	72	–	Dystonia, tremor, gaze palsy, tics
	III.3	Cousin	M	NA	71	–	Dystonia, tremor, gaze palsy, tics
	III.6	Cousin	F	NA	63	–	Tremor
	III.7	Cousin	M	NA	60	–	Likely PSP
	III.9	Cousin	F	NA	52	–	Tremor
	III.11	Sister	F	53	59	–	PSP
	III.20	Cousin	F	63	71	–	Parkinsonism
	III.26	Cousin	M	59	NA	–	Likely PSP
3	III.1	Proband*	F	60	–	72	PSP
	II.3	Aunt	F	70	–	86	Likely PSP
	II.4	Aunt	F	70	–	74	Dementia
4	III.1	Proband*	F	62	–	71	PSP
	II.6	Aunt	F	91	–	96	Dementia
	II.7	Aunt	F	73	–	78	Likely PSP
5	III.5	Proband	F	75	86	–	PSP
	III.4	Brother	M	75	–	86	PSP
	II.2	Mother	F	73	–	83	Likely PSP
6	III.5	Proband	F	37	41	–	PSP
	II.3	Mother	F	41	–	45	PSP
	III.1	Sister	F	37	–	41	Likely PSP
7	III.1	Proband	M	55	59	–	PSP
	II.4	Father	M	70	–	83	PSP
8	II.1	Proband	M	49	68	–	PSP
	I.1	Father	M	NA	–	80	Likely PSP
9	II.1	Proband	F	67	69	–	PSP
	I.2	Mother	F	75	–	83	Likely PSP
10	III.1	Proband	F	70	77	–	PSP
	III.2	Brother	M	70	76	–	Dementia
	III.6	Brother	M	60	64	–	PSP
11	III.6	Proband	F	43	–	48	PSP
	II.3	Father	M	40	–	47	Likely PSP
	III.5	Brother	M	48	–	53	PSP
12	III.3	Proband	F	71	72	–	Likely PSP
	III.5	Brother	M	63	68	–	PSP
	II.4	Father	M	NA	–	49	Gait disorder

M = male; F = female; NA = not available; PSP = progressive supranuclear palsy. *Pathological confirmation.

learning suggestive of abnormal frontal function. He had limitation of voluntary eye movements in all directions, limb rigidity, hypokinesia and gait disturbance which was greater when turning. The retropulsion test was negative but he had bilateral Babinski signs. The patient was thought to have PSP. He informed us that two of his brothers had diplopia, but they have not been examined yet.

Family 2

Clinical data from the proband and other family relatives (individuals 2.III.10, 2.I.1, 2.II.1, 2.II.3, 2.II.5) have been reported elsewhere (de Yébenes *et al.*, 1995). More recently we have personally examined 25 additional family members. The most important new findings are described below.

Individual 2.II.7. He died at the age of 77. His relatives said that he had 'the same' neurological syndrome as his brothers. He had parkinsonism, proven by pictures, and micrographia as shown by samples of his handwriting.

Individual 2.III.1. The 73-year-old female was a cousin of the proband, who suffered from postural hand and head

Table 2 *Clinical features*

No.	Akinesia	Gait	Gaze	Dysp.	Dysa.	Axial dystonia	Dementia	Other	Response to L-dopa	Initial diagnosis	Actual diagnosis
Family 1											
II.12	+	+	+	+	+	+	−	6	+	Parkinsonism	PSP*
III.3	+	−	+	+	+	−	+	.	+	Parkinsonism	PSP
Family 2											
III.10	+	+	+	+	+	+	+	.	+	PSP	PSP*
I.1	+	+	+	−	−	−	−	.	NA	NA	Likely PSP
II.3	−	+	+	+	−	−	−	.	NA	NA	Likely PSP
II.1	+	+	+	+	−	−	+	.	NA	NA	PSP
II.5	+	+	+	+	+	−	+	.	NA	NA	PSP
II.7	+	+	−	−	−	−	−	.	NA	NA	PSP
III.1	+	−	−	−	−	−	−	1	NA	Tremor	Tremor
III.2	−	−	+	−	−	−	−	1, 2, 3	NA	Dystonia	Dystonia, tremor gaze palsy, tics
III.3	−	−	+	−	−	−	−	1, 2, 3	NA	Tremor, dystonia	Dystonia, tremor gaze palsy, tics
III.6	−	−	−	−	−	−	−	1, 4	NA	Tremor	Tremor
III.7	+	+	+	−	−	−	−	1	NA	Tremor	Likely PSP
III.9	−	−	−	−	−	−	−	1	NA	Tremor	Tremor
III.11	+	+	+	+	−	+	−	6	+	Parkinsonism	PSP
III.20	+	+	−	+	−	−	−	1	NA	NA	PK
III.26	+	−	+	−	+	−	−	1	NA	NA	Likely PSP
Family 3											
III.1	+	+	+	+	+	+	+	2, 5, 6	+	Binswanger	PSP*
II.3	+	+	−	−	+	−	−	1	NA	Parkinsonism	Likely PSP
II.4	−	+	−	−	−	−	+	.	NA	Dementia	Dementia
Family 4											
III.1	+	+	+	−	+	−	−	.	−	Parkinsonism	PSP*
II.6	−	+	−	−	−	−	+	.	NA	Dementia	Dementia
II.7	+	+	−	+	+	−	−	.	NA	Parkinsonism	Likely PSP
Family 5											
III.5	+	+	+	−	+	+	+	6, 7	NA	PSP	PSP
III.4	+	+	+	+	+	−	+	2	−	PSP	PSP
II.2	+	+	−	+	−	−	−	.	NA	NA	Likely PSP
Family 6											
III.5	+	+	+	+	+	+	+	5, 6	−	PSP	PSP
II.3	+	+	+	+	+	−	−	1	NA	Parkinsonism	PSP
III.1	+	+	+	−	+	−	−	5	−	Parkinsonism	Likely PSP
Family 7											
III.1	+	+	+	+	−	+	+	5	NA	PSP	PSP
II.4	+	+	+	+	−	+	+	1, 5	−	Parkinsonism	PSP
Family 8											
II.1	+	+	+	+	+	+	+	1	+	Parkinsonism	PSP
I.1	+	+	−	+	−	−	+	.	NA	Dementia	Likely PSP
Family 9											
II.1	+	+	+	−	+	+	−	6	NA	PSP	PSP
I.2	−	+	−	+	+	−	+	1	+	Parkinsonism	Likely PSP
III.1	+	+	+	+	+	+	−	5, 6	−	PSP	PSP
Family 10											
1 III.2	−	−	−	−	−	−	+	.	NA	Dementia	Dementia
III.6	+	+	+	−	+	−	−	2	−	Gait disorder	PSP
Family 11											
III.6	+	+	+	+	+	−	−	.	NA	PSP	PSP
II.3	−	+	+	−	−	−	−	.	NA	CJD	Likely PSP
III.5	+	+	+	+	+	−	−	.	NA	PSP	PSP
Family 12											
III.3	+	+	+	−	−	−	−	.	−	NA	Likely PSP
III.5	+	+	+	+	+	+	+	1, 6	−	Neurodeg. dis.	PSP
II.4	−	+	−	−	−	−	−	.	NA	NA	Gait disorder

Gait = gait disturbance; gaze = supranuclear gaze palsy; dysp. = dysphagia; dysa. = dysarthria; + = present ; − = absent; 1 = tremor; 2 = cranial dystonia; 3 = tics; 4 = orofacial dyskinesia; 5 = apraxia of eyelid opening; 6 = limb dystonia; 7 = myoclonic jerks; PSP = progressive supranuclear palsy; CJD: Creutzfeldt–Jacob disease; neurodeg. dis. = neorodegenerative disease; NA = not available. *Pathological confirmation.

tremor, compatible with essential tremor and mild bradykinesia. At present she is considered not to have PSP.

Individual 2.III.2. The 72-year-old female had cranial dystonia (blepharospasm and dystonia in the lower half of the face), postural tremor, facial tics and vertical supranuclear gaze palsy. Her neuropsychological examination was normal.

Individual 2.III.3. The 71-year-old male had blepharospasm and oromandibular dystonia, facial and phonic tics, vertical upward gaze palsy, predominantly axial rigidity and postural tremor in the right arm. His oculonystagmographic examination revealed slow vertical saccades, with a marked decrease in range and precision, both with predictable and random stimuli, and breakdown of optokinetic nystagmus in the vertical plane. His neuropsychological testing was normal.

Individual 2.III.6. The 63-year-old female had orofacial dyskinesia, increased blink rate, postural tremor in the left arm, and axial and right arm rigidity. Her mental status and gaze were normal.

Individual 2.III.7. The 60-year-old male had bruxism, akinesia and rigidity predominantly in the left arm and leg and postural tremor in the arms. He also had exophoria and failure of convergence. His mental status was normal.

Individual 2.III.9. The 52-year-old female had postural tremor for several years in the upper limbs and a diminished left arm swing. Neuropsychological testing and oculonystagmographic analysis were normal.

Individual 2.III.11. This was a sister of the proband who was diagnosed as having Parkinson's disease at age 53 when she required medical attention for a slowly progressive akinetic rigid syndrome without tremor. She was treated with small doses of L-dopa and her symptoms improved greatly, although she developed visual hallucinations. At the age of 57 she developed typical wearing off fluctuations and at age 59 she complained of swallowing problems which were accompanied by severe weight loss. On examination she had generalized slowness, difficulty in convergence, anterocollis and limb dystonia in the four extremities, a persistent glabellar tap reflex, brisk sustained palmomental responses, hyperreflexia and extensor plantar reflexes. She developed increasing gait difficulty with frequent freezing.

Individual 2.III.20. This patient complained of head tremor at age 63 and progressive slowness and tremor in her right arm. At age 71 she is still active, but with moderate right side akinesia and rigidity, mild walking difficulty, dysphagia and urinary incontinence.

Individual 2.III.26. At age 59 he complained of tremor at rest in the right hand, hypophonia and excessive sweating. During the following year he developed an akinetic syndrome with gait disturbance, hypomimia, generalized rigidity, dystonia in the lower face and supranuclear gaze palsy with slowing of horizontal and vertical saccades in the oculonystagmographic study. Cognitive testing was normal.

Family 3

Proband, individual 3.III.1. This 72-year-old female developed a lack of initiative, lack of social interest and apathy at age 60. One year later she had speech abnormalities and gait disturbance with frequent falls. She had hypertension and a history of smoking. Her neurological examination at age 64 revealed hypomimia, bradykinesia, hyperreflexia and gait disturbance and she was diagnosed as having Binswanger's disease. Her symptoms worsened slowly over the following years, her walking became progressively worse and she developed limitation of downgaze. Two years later she complained of dysphagia and urinary incontinence. At age 69 she was treated with L-dopa, which produced a mild, transient improvement, and with dopamine agonists, without improvement. She was examined at age 70 when there was evidence of frontal lobe dysfunction, severe dysarthria and dysphagia, facial dystonia and apraxia of eyelid opening. The oculomotor examination revealed limitation of downgaze, difficulty in convergence and slowing of horizontal and vertical saccades. Her neurological examination revealed rigidity and akinesia predominantly in her left limbs, retrocollis and left lower limb dystonia. Her stretch reflexes were exaggerated and she had left foot dystonia. Investigation of her regional cerebral blood flow by HMPAO-SPECT (single photon emission tomography) revealed a deficit of perfusion in the frontal region. A cerebral MRI showed midbrain and subcortical atrophy which was more severe in frontal region. During the ensuing months she became wheelchair bound, unable to read because of complete vertical and horizontal gaze palsy and she developed severe dysphagia and oromandibular dystonia but she declined gastrostomy. She died of pneumonia at age 72. Macroscopic examination of the brain was normal except for pallidal atrophy with brownish discoloration and loss of pigment in the substantia nigra. Light microscopy revealed neuronal loss, gliosis and high density of neurofibrillary tangles and neuropilic threads with positive immunoreactivity to tau and PHF-1 (paired helical filaments) in globus pallidus, subthalamic nucleus, substantia nigra, peri-aqueductal region and oculomotor complex. There was a moderate number of tangles in caudate, putamen, locus coeruleus, pontine nuclei, inferior olive, nucleus ambiguus, raphe nuclei and dentate nucleus.

Individual 3.II.3. This was the proband's aunt on whom we only have retrospective information from relatives. Photographs of the patient taken during her youth and early adult life did not reveal any neurological disease. She developed a parkinsonian syndrome around age 70 with tremor in her hands, early gait disturbance with frequent falls

and speech difficulties. She developed progressive and severe deterioration of her gait and speech and became bedridden and virtually mute for the last few years before her death at age 86.

Individual 3.II.4. This was the proband's aunt on whom we only have reports from relatives. In the last years of her life she developed dementia with frequent hallucinations and severe gait disturbance and she died at age 74.

Family 4

Proband, individual 4.III.1. This was a 62-year-old woman with a history of hypertension, diabetes and smoking who developed progressive gait disturbance and speech difficulty. One year later her neurological examination revealed dysarthria with orolingual apraxia, and persistent glabellar tap and palmomental reflexes. Her mental status was normal. She had ocular dysmetria, slowing of saccades and breakdown of vertical opticokinetic nystagmus. Standing and tandem walking were difficult. She was treated with L-dopa without improvement and at age 65 she lost vertical gaze (up and down) with abolition of vertical optokinetic nystagmus. She had axial and limb rigidity, facial dystonia, bradykinesia and loss of balance and gait difficulty. A cerebral MRI revealed corticosubcortical and brainstem atrophy, mostly in the mesencephalic tegmentum. She died at age 71 and had a post-mortem examination. Light microscopy revealed neuronal loss, gliosis and presence of neurofibrillary tangles and neuropil threads in neurons and glia in globus pallidus (more severe in the medial segment), frontal cortex, subthalamic nucleus, substantia nigra, red nucleus, oculomotor complex, locus coeruleus, dentate and inferior olivary nucleus. Tangles were also identified in Meynert's nucleus, hippocampus, subiculum and pons.

Individual 4.II.6. This was the proband's aunt for whom we only have reports from relatives. In the last years of her life she had a neurological syndrome characterized by dementia, gait disturbance and severe weight loss. She died aged 96 years.

Individual 4.II.7. This was the proband's aunt and a sister of 4.II.6 for whom we only have reports from relatives. She was said to have Parkinson's disease in her seventies. She moved slowly, had no tremor, had frequent falls backwards, developed severe dysphagia and dysarthria and died at age 78.

Family 5

Proband, individual 5.III.5. This 75-year-old female had a progressive syndrome characterized by slowness, clumsiness and dementia. Her neurological examination at age 83 revealed facial dystonia, dysarthria, rigid-akinetic syndrome and gait disturbance. A cerebral MRI revealed corticosubcortical atrophy. During a visit to her home when she was 85 years old, a neurological examination revealed disorientation, abnormalities of memory, limitation of vertical gaze, generalized rigidity (predominantly axial), retrocollis, facial and upper limb dystonia. She was unable to walk and she had a spontaneous tendency to fall backwards. Occasionally she had myoclonic jerks in the arms. She is still alive at age 86.

Individual 5.III.4. This was the proband's brother and his neurological disorder began at age 75 with an akinetic rigid syndrome, dementia, dysphagia, dystonia (antero- and retrocollis) and blepharospasm. His neurological examination 10 years later revealed hypokinesia, upgaze abolition, reduction of the verbal fluency and axial and limb rigidity. A cerebral MRI revealed corticosubcortical and brainstem atrophy. He was unable to walk, developed severe dysphagia and he lost 12–15 kg. He died at the age of 86. During the last few months of his life he took L-dopa without apparent improvement.

Individual 5.II.2. This was the mother of the previous patients for whom we only have information from relatives. For several years she had a neurological syndrome similar to that suffered by her children before dying aged 83.

Family 6

Proband, individual 6.III.5. At age 37 she developed generalized slowness, gait disturbance with frequent falls and micrographia. Her neurological examination 1 year later revealed dysarthria, difficulty in convergence, abolition of upgaze and apraxia of eyelid opening. Stretch reflexes were exaggerated, the left plantar response was extensor and she had axial rigidity and right arm dystonia. She was treated for 3 months with L-dopa without improvement and in the following years she developed axial dystonia, vertical gaze palsy and slow horizontal saccades. Her dysphagia became so severe that she lost weight and required percutaneous gastrostomy. She became virtually mute only being able to express affirmation or negation with slow movements of her hand. Cognitive evaluation was difficult but she was suspected by her physicians to have frontal lobe dementia. She is now 41 years old.

Individual 6.II.3. This was the proband's mother whose neurological disorder began at age 41 with tremor in the left arm and progressive slowness. She was diagnosed with Parkinson's disease 2 years later. When she was 44 years old she had hypophonia, dysphagia and gait disturbance with frequent falls. Neurological examination revealed rigidity, exaggerated stretch reflex, left extensor plantar response and micrographia. A right thalamotomy was performed without improvement. One year later her left arm was fixed in flexion and pronation, she had severe generalized bradykinesia and extensor plantar responses. A few months later there was evidence of voluntary upgaze paralysis and she died at age 45.

Individual 6.III.1. This was the proband's sister who developed a progressive akinetic syndrome when she was 37 years old. Two years later she had a gait disturbance with frequent falls and micrographia and neurological examination revealed hypophonia, a reduced range of vertical gaze movements, difficulty for ocular convergence, apraxia of eyelid opening, exaggerated stretch reflexes and axial rigidity. She was treated with bromocriptine without improvement and died aged 41.

Family 7

Proband, individual 7.III.1. A 55-year-old male presented with loss of memory and progressive gait disturbance, frequent backwards falls and dysphagia, and neurological examination 3 years later revealed downgaze limitation. He is now 59 years old and has an akinetic rigid syndrome with severe gait disturbance, retrocollis, apraxia of eyelid opening and downgaze limitation with occasional diplopia.

Individual 7.II.4. This was the proband's father who, during the last 13 years of his life, had an akinetic rigid syndrome with postural tremor in the left arm, dementia with apathy, gait disturbance, dysphagia and significant weight loss. Neurological examination revealed retrocollis, apraxia of eyelid opening and micrographia. He was treated with L-dopa without improvement. Later he lost vertical downgaze and developed diplopia. He died at age 83.

Family 8

Proband, individual 8.II.1. This was a 49-year-old male who developed bradykinesia in his left arm. He was diagnosed with Parkinson's disease and was treated with L-dopa and bromocriptine with clear improvement for many years. When we saw him for the first time at age 63 he was hallucinating and examination revealed hypokinesia, bilateral rest and postural tremor, somnolence and fatigue. Two years later he had frequent falls, dysarthria and memory loss and at age 66 the akinetic syndrome had progressed and he had horizontal gaze limitation, difficulties in convergence and 'frontal dementia'. After 15 years of L-dopa therapy his akinesia was still improved but now at age 68 he has dysphagia, facial dystonia, retrocollis, supranuclear gaze palsy affecting all directions and gait disturbance.

Individual 8.I.1. This was the proband's father on whom we only have reports from relatives. He had an akinetic rigid syndrome without tremor with frequent falls and dementia. During the last years of his life he had dysphagia with weight loss and he died at age 80.

Family 9

Proband, individual 9.II.1. At the age of 67 this woman had a predominantly right-sided akinetic rigid syndrome. Two years later she developed dysarthria, palilalia, facial dystonia and axial and right arm dystonia. Vertical ocular movements were abolished and horizontal ones were slow. She developed urinary incontinence and loss of balance with frequent backwards falls.

Individual 9.I.2. This was the proband's mother who, at age 75, developed loss of balance, gait disturbance, postural tremor, dementia, dysarthria and severe dysphagia with weight loss. She received treatment with L-dopa with clear improvement, but she had hallucinations. She died at age 83.

Family 10

Proband, individual 10.III.1. At the age of 70 this lady developed unexplained falls. Her balance progressively deteriorated with frequent falls and she also had micrographia and general slowness of movement. By the time we examined her 7 years after symptom onset, she was unable to stand unaided but was able to walk with the aid of two people, with a tendency to fall backwards. Her neck was fixed in an anteroverted position and she had a complete vertical supranuclear palsy with slow and hypometric lateral saccades, blepharospasm and apraxia of eye lid opening. She had a low pitched dysarthria and mild symmetrical distal bradykinesia and rigidity. There was intermittent dystonic posturing of the right leg with extension at the knee and plantar flexion at the ankle.

Individual 10.III.2. At the age of 70 this man developed nocturnal restlessness and was frequently found by his wife wandering around the house. He developed urinary frequency and mild short-term and remote memory loss. At the age of 76 he was unable to answer any questions or follow commands but he had dressing apraxia and required assistance for feeding. He walked unaided with steady balance as he turned and his eye movements were normal. Although he could not be formally examined there was no obvious bradykinesia, but there was mild symmetrical cogwheel rigidity, together with mild axial rigidity and turning and sitting *en bloc*. There were small amplitude irregular, stimulus sensitive finger movements indicating cortical myoclonus. He developed marked bradykinesia and rigidity following a depot injection of anti-psychotic medication.

Individual 10.III.6. At the age of 60 this man developed difficulty walking with unsteadiness and backwards falls while pushing a wheelbarrow. Over the following year he noted that he had difficulty in starting to walk with his feet 'glued to the floor' and had difficulty in turning, particularly in confined spaces, although he walked with normal step size. He also developed mild micrographia. At this time he had no bulbar symptoms and examination of his extraocular movements was normal. His cranial MRI showed no vascular changes and the diagnosis was of a frontal gait disorder. Four years after symptom onset he developed mild dysarthria

and difficulty with reading. Examination showed that he had developed mild slowing of vertical saccadic eye movements and blepharospasm.

Family 11

Proband, individual 11.III.6. At the age of 43 this lady developed a lack of interest in day-to-day events and chronic insomnia. She had slowing of her speech, difficulty in shifting her gaze and she became obsessional about performing particular tasks. Two years after symptom onset she had impairment of postural reflexes and a complete absence of vertical saccadic eye movements to command but well preserved smooth pursuit eye movements. The mini-mental test score was 30/30 but there was impaired verbal fluency. Over the next few years she developed increasingly severe dysarthria and dysphagia and died of a respiratory infection 5 years after symptom onset.

Individual 11.II.3. In his forties this man developed 'problems with his eyes', gait unsteadiness and frequent falls, some of them quite serious. He also found it hard to judge distances and frequently bumped into people, and he is recalled by his family as having had staring eyes. He deteriorated quite rapidly, was transferred to a nursing home and then to a hospital where he died in 1954 at the age of 47 years. The death certificate diagnosis was of Creutzfeldt–Jacob disease but no pathological report has been ever found. Both his children are now dead and we have never obtained medical records or the post-mortem details on him. However, from accounts given by his daughter and members of the broader family that this patient had the same staring eyes and other neurological features observed in his children we feel that there is sufficient grounds to be suspicious of PSP.

Individual 11.III.5. He was a professor of economic geography who, in his forties, was noted by his work colleagues to become obsessive and worried about small details. At the age of 48 he developed difficulty in focusing and then in reading and started to have frequent falls and memory loss, particularly for recent events. When seen by a neurologist in 1989 he had symmetric akinetic–rigid syndrome with severe supranuclear downgaze palsy, slurring of speech, marked hypomimia, diffuse pyramidal signs, a brisk jaw jerk and bilateral grasp reflexes. At the age of 52 he had a severe supranuclear gaze palsy and was profoundly bradykinetic and dysarthric with marked postural instability. There were no sensory or cerebellar signs and he scored 25/30 on the Mini-Mental State Examination. All laboratory tests were normal and L-dopa was tried without benefit. He became mute and increasingly immobile and died at the age of 53.

Family 12

Proband, individual 12.III.3. This right-handed woman started to fall at 71 and she also complained of unsteady gait and had difficulty looking down, particularly when going down stairs or eating . Anti-parkinsonian therapy was initiated but with no benefit. On examination she had a 'dystonic, pseudobulbar' face, markedly impaired vertical gaze (supranuclear), impaired vertical pursuit, hypometric saccades and square wave jerks. On motor examination there was bradykinesia, tone was normal and there was no cogwheeling. Stretch reflexes were exaggerated but the plantar responses were down going. Her gait was narrow-based and unsteady with unpredictable falls and there was limited arm-swing and she turned en bloc. She is still alive.

Individual 12.III.5. This man began having symptoms at the age of 63 with personality changes and increasing irritability. He subsequently developed dysarthria followed by falls and an unsteady gait. One year later a 'wide-eyed' stare was evident and he later developed coarse rhythmical activity and posturing in his right hand. The family noted rigidity and dysphagia as well as cognitive changes (poor memory and judgement) but he did not benefit from L-dopa. On examination he had typical PSP facies and there was also hypophonia and fluctuating dysarthria. He had impaired vertical gaze and pursuit with a normal oculocephalic reflex. There was left facial weakness. On motor examination there was bradykinesia, axial dystonia and dystonic posturing of his right hand. There was also a coarse, intermittent tremor of his right hand and gegenhalten was present in the legs. He had a persistent glabellar tap and grasp reflex, rapid alternating movements were severely impaired, there was axial dystonia and his gait was unsteady. He is still alive.

Individual 12.II.4. This man died at the age of 49 years from liver disease. He had gait problems with frequent falls.

Clinical genetics

In these families we have found 22 secondary PSP cases among 133 first and second degree relatives from whom there was available information. All the patients with 'likely PSP' (n = 12) had supranuclear gaze palsy and/or frequent falls or gait disturbance early in the course of the disease. Other individual members of these families presented with other neurological disorders including isolated tremor (n = 3), dementia (n = 3), parkinsonism (n = 1), gait disturbance (n = 1), and tremor, dystonia, gaze palsy and tics (n = 2).

In these families we found 34 individuals with clinical or pathological criteria of PSP (12 probands and 22 familial cases). Seventeen were male and 17 female and the mean age at onset of symptoms was 59.9 ± 12.4 years (n = 28). In 17 of these patients there was information available about vascular risk factors: five (29.41 %) were heavy smokers, two had high blood pressure (11.76 %) and two diabetes (11.76%). The duration of the disease from clinical onset to death was 8.81 ± 3.76 years (n = 16). Bradykinesia was the presenting feature in 15 patients, gait disturbance in five

Table 3 *Clinical features in 34 individuals with PSP*

	n	%
Bradykinesia	31	91
Gait disturbance	32	94
Supranuclear gaze palsy	29	85
Dysphagia	22	65
Dysarthria	21	62
Axial dystonia	12	35
Dementia	14	41
Tremor	8	23
Apraxia of eyelid opening	6	18
Blepharospasm	2	6
Limb dystonia	8	23
Myoclonic jerks	1	3
Response to L-dopa	7	44*

*Percentage of 16 patients who received L-dopa.

and change in personality in three. In nine patients it was not possible to determine the initial clinical symptom.

The frequency of the different clinical symptoms is summarized in Table 3. Gait disturbance was the most frequent alteration during the progression of disease (94% of the patients). Seven out of 16 patients treated with L-dopa improved initially (three out of the four cases confirmed by pathology) and in three of them this response was sustained for more than 3 years. In one patient, L-dopa therapy was thought to be of marginal efficacy, but its discontinuation because of paralytic ileus produced a malignant catatonic syndrome that caused his death in spite of intravenous administration of lisuride.

In order to evaluate if there were any differences between familial and sporadic PSP we compared clinical features of the five probands from the families seen in Fundación Jiménez Díaz with the last five cases of sporadic PSP seen by us in the same hospital (Table 4). We did not find clinical differences between familial and sporadic PSP in terms of age at onset and initial clinical findings. Patients with familial PSP more frequently had dystonia which may have reflected the more prolonged follow-up.

Discussion

This study presents a set of clinical data and clinical genetics of familial PSP. In 12 families from Europe and North America we found 22 secondary cases compatible with PSP in addition to the 12 probands, all with typical clinical features. Four of the probands had neuropathological confirmation of the diagnosis of PSP. In these families there were relatives with other neurological disorders including tremor (three patients), adult onset focal dystonia, tremor, gaze palsy and tics (two patients), dementia (three patients), parkinsonism (one patient) and gait disorder (one patient). Most of these disorders are frequent in the general population and, therefore, it is not possible to conclude whether these disorders appeared in these families by chance or as oligosymptomatic manifestation of PSP.

The recognition of familial cases of PSP requires (i) intensive investigation of the families including field trips and home visits of apparently healthy family relatives and (ii) long-term care and follow-up of the proband and relatives by specialized neurologists. These methods of evaluation and long-term follow-up are not common in our health care systems. For example, patient 1.III.12 was thought to be a sporadic case of PSP during her lifetime, although an intensive search for other cases in the family was performed. However, 4 years after the proband's death a secondary case appeared in her family. Patient 2.III.10 was thought to have sporadic PSP during his lifetime. He had a sister who at first was considered to have idiopathic, L-dopa responsive, Parkinson's disease but 1 year after the proband's death, his sister developed severe dysphagia, weight loss and opthalmoplegia. Four years later a first cousin developed akinesia, apathy, cranial dystonia and gaze palsy. After interviewing around 30 family relatives we concluded that five deceased ancestors considered, during their lives, to have 'parkinsonism', 'dementia', 'senility' or 'cerebrovascular disease' in fact had PSP. Patient 7.III.1 was thought to have idiopathic Parkinson's disease. His attending neurologist was not told that the patient's father died of 'parkinsonism'. Interviews with the patient's mother and brother, in addition to a review of the records and family pictures, provided convincing evidence of PSP.

The pattern of inheritance is compatible with autosomal dominance with reduced penetrance. Lack of evidence for vertical transmission in three or more generations, except in one family, could be related to insufficient information about grandparents of elderly patients, most of them with some kind of cognitive impairment. There are, however, other explanations. The disease was only described 3 decades ago (Steele *et al.*, 1964) and people lose contacts with their place of origin because of the strong migratory movements that have taken place in many parts of the world, and the increased life expectancy of around 25 years that has occurred during this century. Since the prevalence of PSP is age related (Daniel *et al.*, 1995) the chances of developing the disease in gene carriers is now much greater than at the beginning of the century.

The proportion of affected individuals in different generations increases with age. The number of secondary cases in the generation of the probands (6 out of 37 siblings and 3 out of 34 cousins, i.e. 9 out of 71) was smaller than the number of secondary cases in the previous generation (7 out of 24 parents and 5 out of 37 uncles and aunts, i.e. 12 out of 61) (Table 3). This is not surprising since the mean age of the siblings is 53 years and the age adjusted annual incidence of PSP increases from 1.4 at age 50 to 14.2 per 100 000 inhabitants at an age higher than 80 years (Bower *et al.*, 1997). Therefore, it is likely that some living relatives will develop the disease in the future.

The diagnosis of PSP is difficult in patients with atypical clinical phenotypes. Confirmed cases of PSP by autopsy have been diagnosed in life as Parkinson's disease, corticobasal

Table 4 *Clinical features in sporadic and familial PSP*

	Sporadic (n = 5)	Familial (n = 5)
Age of onset*	59.2 ± 6.8	60.2 ± 6.2
Smoking	2	4
High blood pressure	2	1
Other	1 diabetes mellitus	1 high cholesterol levels
Presenting feature	4 gait disorder, 1 cognitive changes	3 bradykynesia, 1 gait disorder, 1 cognitive changes
Bradykinesia	5	5
Gait disturbance	5	5
Supranuclear gaze palsy	5	5
Dysphagia	3	4
Dysarthria	4	5
Axial dystonia	1	5
Dementia	4	3
Tremor	1	1
Apraxia of eyelid opening	1	1
Blepharospasm	1	0
Limb dystonia	0	3

*Mean ± SE.

degeneration, multisystemic atrophy or Alzheimer's disease (Jackson *et al.*, 1983; Boller *et al.*, 1989; Rajput *et al.*, 1991; Hughes *et al.*, 1992). The pathology of PSP is characterized by a variable combination of neuronal loss, gliosis and neurofibrillary tangles in many brain areas including the cerebral cortex, basal ganglia and the brainstem (Hauw *et al.*, 1994; Daniel *et al.*, 1995). Although typical cases are characterized by akinesia, supranuclear gaze palsy, rigidity, axial dystonia, gait disturbance and dementia, there are patients with atypical symptoms and typical pathology (Daniel *et al.*, 1995). In some of these patients the diagnosis of PSP could be easily missed.

Improvement with L-dopa is considered typical of Parkinson's disease and rare in PSP to the point that lack of response is considered support criteria for the diagnosis of PSP (Jellinger, 1995). However, 35–50% of the patients improve their bradykinesia or rigidity with L-dopa (Nieforth and Golbe 1993; Litvan *et al.*, 1997). In the present study we found that 7 out of 16, 44% of the treated patients, improved with this treatment. Furthermore, three out of the four cases confirmed by pathology responded to L-dopa. In most of the patients the response was modest and transient, but in three individuals (1.III.3, 2.II.11 and 8.II.1) the benefit was maintained for several years. These patients were considered to have Parkinson's disease before they developed the complete typical syndrome of PSP. The possibility that some of these patients had Lewy body pathology cannot be ruled out in spite of the lack of Lewy bodies in the three patients with pathological confirmation of PSP and response to L-dopa. Unfortunately, we could not obtain post-mortem evaluation of some of the relatives of the probands with atypical clinical findings but [^{18}F]dopa and deoxyglucose-PET scans have revealed abnormal data compatible with PSP in members of these families with atypical phenotypes or who are clinically asymptomatic (Piccini *et al.*, 1998).

Genetic risk factors for PSP are uncertain. In a case-control study by means of questionnaires answered by 50 patients it was found that parkinsonism (odds ratio 5.0) and dementia (odds ratio 3.6) were more common among first degree relatives of PSP patients (Davis *et al.*, 1988) but, due to the small size of the cohort, the difference was not significant. Recent reports of familial PSP have raised the possibility of a genetic factor in the cause of this disease (David *et al.*, 1968; Mata *et al.*, 1983; Ohara *et al.*, 1992; Brown *et al.*, 1993; Gazely and Maguire, 1994; Tetrud *et al.*, 1994; de Yébenes *et al.*, 1995; Golbe *et al.*, 1995; Lanotte *et al.*, 1996; Tetrud *et al.*, 1996). The pattern of inheritance in these previous reports was variable. Four of these families had affected members in two or more generations, suggesting autosomal dominant transmission, while five families had affected individuals in the same generation and consanguinity was observed in one. So far, familial clustering PSP has been reported in 20 families including those in the present study. There is evidence for vertical transmission in 10 families, consanguinity in one and horizontal aggregation in 13 families. Considering the difficulties in the diagnosis it is likely that the number of familial aggregates of PSP is greater than previously thought, suggesting that PSP could be a hereditary disorder, at least in some families.

Recent reports (Conrad *et al.*, 1997; Lazzarini *et al.*, 1997) have described a higher prevalence of the A_0 polymorphism of the gene for tau in patients with sporadic PSP than in the general population. Higgins and colleagues (Higgins *et al.*, 1998) confirmed these data and suggested that familial PSP could be inherited as an autosomal recessive disorder linked to the tau gene. These observations are interesting since the pathological lesions found in the brain of patients with PSP are tau containing neurofibrillary tangles. The significance of this finding, however, is very difficult to interpret since homozygosity for the A_0 polymorphism occurs in around 55% of the population while the prevalence of PSP, even in aged individuals, is only 70 out of 100 000 inhabitants.

Furthermore, no linkage has been found (J. Hoenicka, M. Pérez, J. Pérez-Tur, A. Barabash, M. Godoy, R. Astarloa, J. Avila, T. Nyggard, J. G. de Yébenes, unpublished results) in the region 17q 21–22 where the gene for tau is localized, during a genomic search performed in families 2 and 7 of this study. In addition, we found that the polymorphism A_0 has a similar distribution in affected and non-affected members of families 2 and 6 of this study (Hoenicka *et al.*, manuscript in preparation), and no evidence of linkage was found between the PSP phenotype and the gene for tau after analysing the data for both a pattern of autosomal dominant and recessive inheritance in these families.

It is very important that the familial character of PSP is recognized in order to look for additional families that could be included in a genetic search. Finding a gene responsible for PSP could be a great step forward towards finding a valuable treatment for this disease.

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OBSERVATION

A New Mutation of the τ Gene, G303V, in Early-Onset Familial Progressive Supranuclear Palsy

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Background: Progressive supranuclear palsy (PSP) is a clinicopathological syndrome related to τ deposits and in linkage disequilibrium with τ polymorphisms. Some rare familial PSP cases have been related to τ gene mutations.

Objective: To present the clinical, pathological, and molecular data of one family with early-onset autosomal dominant PSP.

Design: We performed clinical examinations, quantitative neurological tests, positron emission tomographic scans with fluorodopa F 18 and raclopride C 11, analysis of τ mutations, neuropathological examinations, and protein analyses on brain specimens.

Results: Three family members had PSP confirmed by pathological features in the proband. A novel mutation of τ, G303V, was found in the proband and other family members. τ Isoforms with 4 microtubule-binding repeats were overexpressed in the proband brain.

Conclusions: The G303V mutation of τ is associated with autosomal dominant PSP. Expression of 4 microtubule-binding repeat τ isoforms is increased in the proband.

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Progressive supranuclear palsy (PSP) is usually sporadic, although some familial cases have been described.[1] Mutations in the τ gene, encoding for the microtubule-associated protein τ, have been mainly related to frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17).[2-4] Frontotemporal dementia with parkinsonism linked to chromosome 17 is characterized by atypical parkinsonism or frontotemporal dementia. Typical cases of PSP and FTDP-17 can be differentiated by clinical symptoms because dementia appears early in FTDP-17 and late or in milder form in PSP. However, atypical familial PSP has also been related to τ gene mutations.[5,6]

We describe herein a family with clinicopathological features of PSP presenting a novel mutation in the τ gene, G303V. Analysis of brain proteins revealed hyperphosphorylation of τ and an increased 4 microtubule-binding repeat (4R)–3 microtubule-binding repeat (3R) ratio of τ isoforms.

METHODS

MOLECULAR ANALYSIS OF THE τ GENE

After informed consent was provided, blood samples were obtained from affected and asymptomatic family members. τ Exons 1 and 9 through 13 were amplified from genomic DNA. Polymerase chain reaction (PCR)–amplified fragments of exon 10 were analyzed by single-stranded conformational polymorphism, and those with abnormal electrophoretic patterns were directly sequenced.

POSITRON EMISSION TOMOGRAPHIC SCANNING

Positron emission tomographic scanning was performed at the Centre d'Exploration et de Recherche Médical par Emission de Positons (Cyclotron Unit) on a tomograph (model TTV 03 LETI; Siemens, Knoxville, Tenn). At positron emission tomographic scanning, the subjects were off dopaminergic medication for at least 12 hours. Fluorodopa F 18 uptake and raclopride C 11 positron emission tomographic scans were analyzed as described.[7,8]

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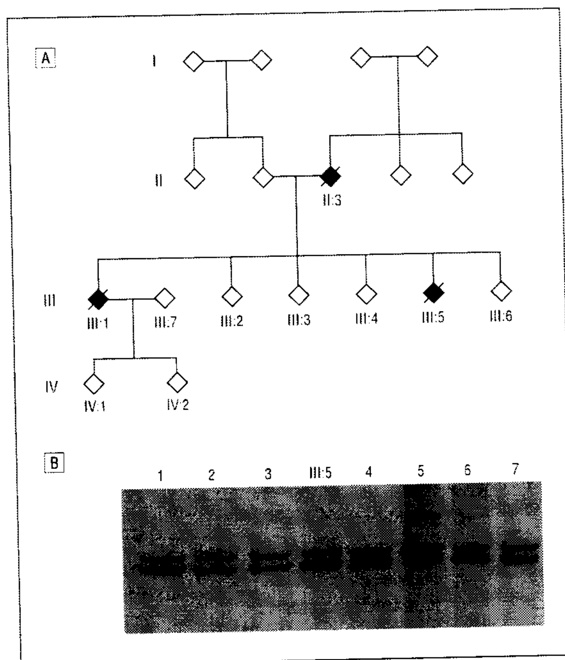

Figure 1. Genealogical tree of family members (A) and single-stranded conformational polymorphism (SSCP) analysis (B). In A, the schematic family tree shows the presence of progressive supranuclear palsy (PSP) in the proband (individual III:5), with a G303V mutation of τ, and in 2 members of the family (II:3 and III:1). In B, SSCP analysis of exon 10 in family members revealed an abnormal band pattern in the proband (III:5) and in individuals 4 through 6, who represent asymptomatic heterozygote carriers. The sex and genealogical order of the members of the family were masked for confidentiality. Shaded symbols with a diagonal line indicate deceased individuals who had PSP; unshaded symbols, healthy individuals.

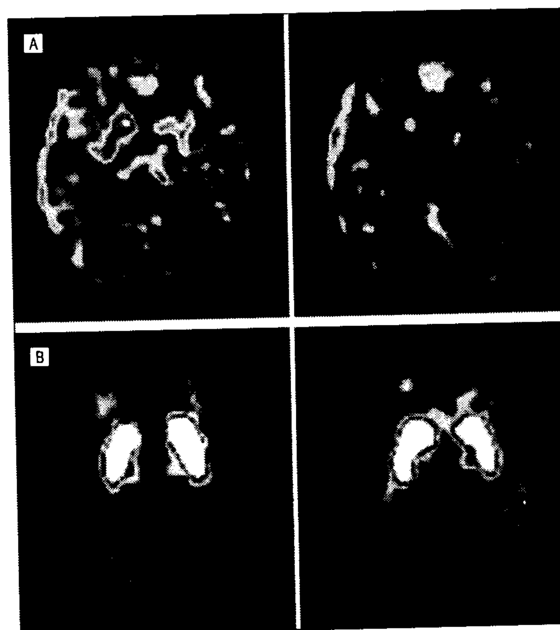

Figure 2. Representative transaxial positron emission tomographic images of the proband: a fluorodopa F 18 scan shows a major reduction of fluorodopa F 18 uptake in the striatum, with clear asymmetry (A; caudate), and raclopride C 11 uptake in the striatum is normal (B; putamen).

NEUROPATHOLOGICAL ANALYSIS

The proband's brain was divided into 2 halves for neuropathological and biochemical analyses. Paraffin-embedded sections were stained with hematoxylin-eosin, Nissl, and Gallyas, and immunostained with a polyclonal antibody against the C-terminal region of human τ (A024; Dako, Trappes, France).

PROTEIN EXTRACTS AND WESTERN BLOTS

Protein samples from the frontal cortex, temporal cortex, striatum (St), and thalamus were obtained from the proband, a patient with Alzheimer disease, and a control subject without dementia. Soluble and particulate protein samples from Sarkosyl extracts,[9] containing aggregated τ phosphoproteins, were separated in sodium dodecyl sulfate–polyacrylamide gels, electroblotted onto nitrocellulose sheets, incubated with specific antibodies, and developed with the ECL system (Amersham, Braunschweig, Germany).

Antibodies used include 7.51, raised against a phosphorylation-independent epitope located at the microtubule-binding region of τ, and PHF-1, recognizing phosphorylated τ at serine positions 396 and 404.

REVERSE TRANSCRIPTION–PCR ANALYSIS OF τ ISOFORMS

The expression of messenger RNA containing or lacking exon 10 was studied by reverse transcription–PCR on complementary DNA samples obtained from striatal samples of the previously mentioned cases.[10]

RESULTS

DESCRIPTION OF THE FAMILY

This family was partially described earlier (**Figure 1**A).[1] Three members were diagnosed as having PSP, according to neuropathological[1,11] or clinical[12] criteria. The clinical features were as follows.

The proband (individual III:5), at the age of 37 years, developed akinetic-rigid syndrome, gait disturbance, frequent falls and micrographia, dysarthria, difficulty in convergence, abolition of upgaze, apraxia of eyelid opening, and exaggerated stretch reflexes with extensor left plantar response. The result of a cognitive examination showed a Mini-Mental State Examination score of 22 of 30 and a Quick Frontal Efficiency Scale score of 11 of 18.

The mean fluorodopa F 18 Ki dopa influx rate constant (I) values were 3.3×10^{-3}min^{-1} (caudate) and 3.2×10^{-3}min^{-1} (putamen) (**Figure 2**A), reduced by 70.8% and 70.9%, respectively, compared with controls. Fluorodopa F 18 uptake was asymmetrical in the patient (Ki values were 4.4×10^{-3}min^{-1} and 2.3×10^{-3}min^{-1} for the right and left sides of the caudate nucleus, respectively, and 3.7×10^{-3}min^{-1} and 2.8×10^{-3}min^{-1} for the right and left sides of the putamen, respectively). Mean raclopride C 11 binding was similar and normal in the Striatum (Figure 2B), with values of 3.5×10^{-3}min^{-1} and 3.8×10^{-3}min^{-1} for the putamen and caudate nucleus, respectively.

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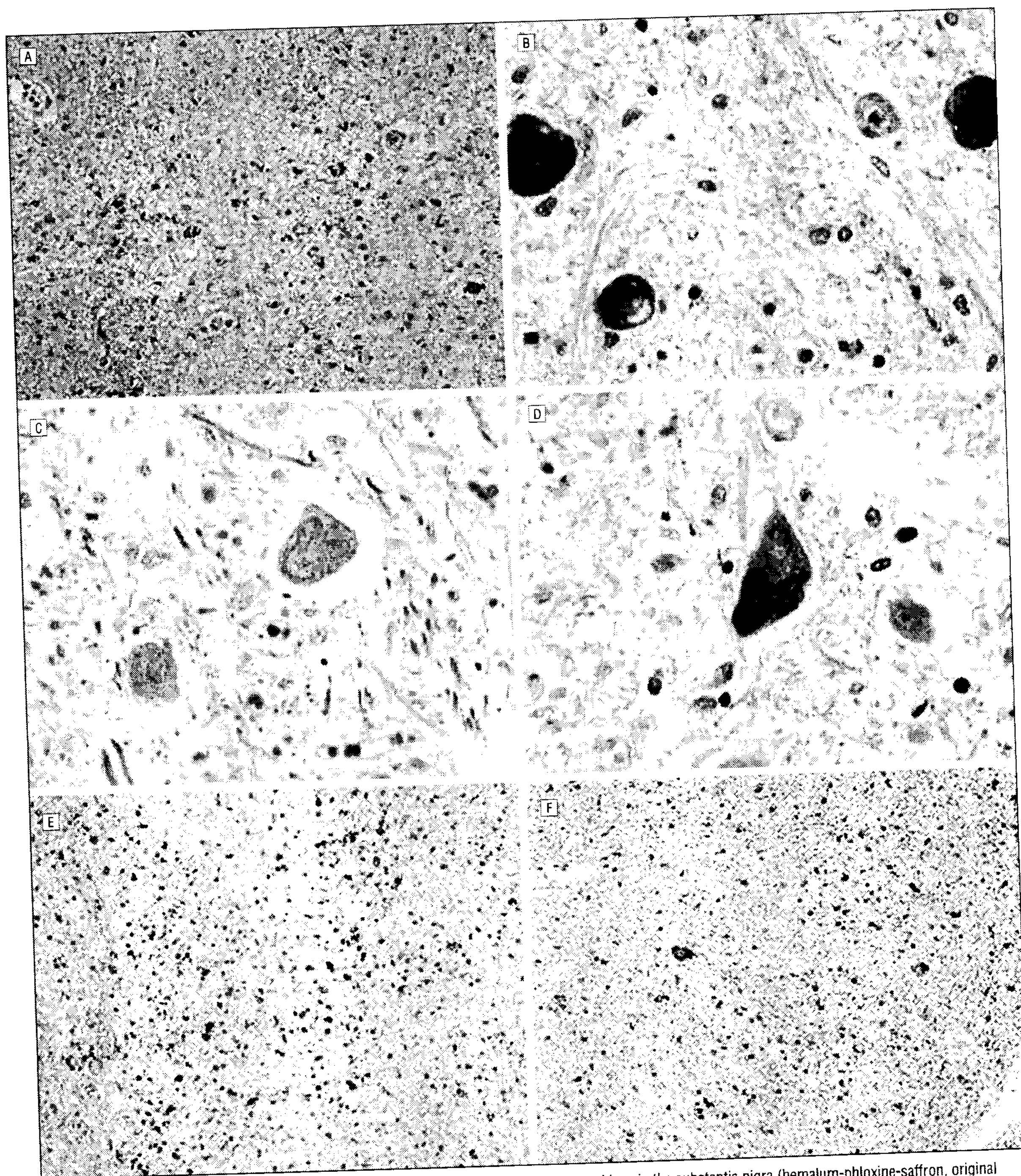

Figure 3. Histological and immunocytochemical properties of the proband brain. A, Neuronal loss in the substantia nigra (hemalum-phloxine-saffron, original magnification ×100). B, Globose appearance of τ-immunoreactive neurofibrillary tangles in the locus coeruleus (τ, original magnification ×400). C, Flame-shaped neurofibrillary tangles in the nucleus of the third cranial nerve (silver, original magnification ×400). D, Flame-shaped τ-immunoreactive neurofibrillary tangles in the nucleus of the third cranial nerve (τ immunoreactivity, original magnification ×400). E, Neuronal and glial cells showing τ immunoreactivity in the globus pallidus (τ immunoreactivity, original magnification ×100). F, Neuronal and glial cells showing τ immunoreactivity in the dentate nucleus of the cerebellum (τ immunoreactivity, original magnification ×100).

This patient worsened slowly, developing axial dystonia, mutism, complete vertical gaze palsy, slow horizontal saccades, severe dysphagia, and weight loss requiring percutaneous gastrostomy. She died at the age of 45 years.

The proband's mother (individual II:3) developed PSP at the age of 41 years and died at the age of 45 years.

The proband's sister (individual III:1) developed PSP in her late 30s and died at the age of 41 years.

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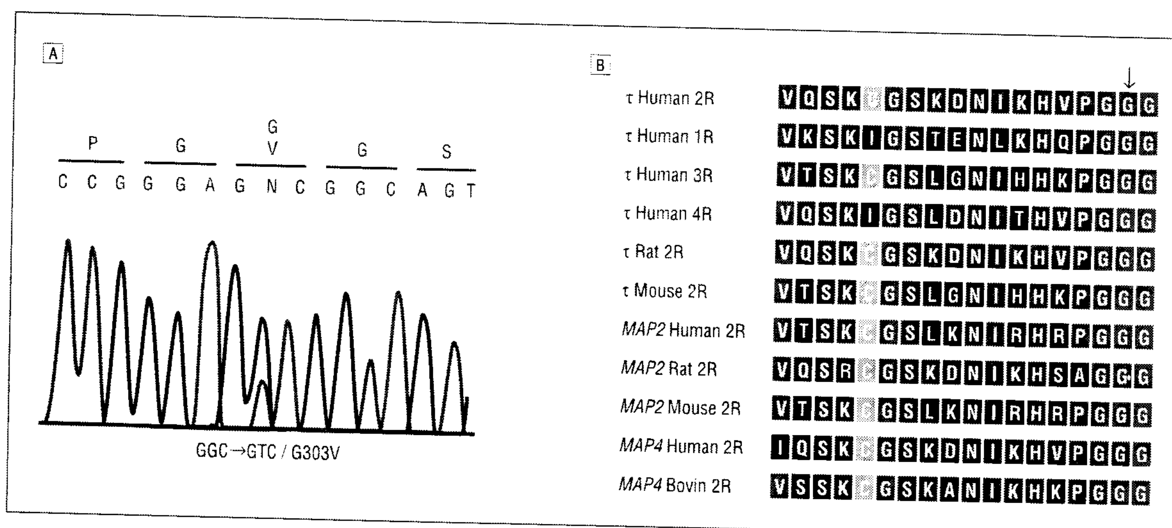

Figure 4. Analysis of a τ gene mutation: a fragment of the complementary DNA sequence reveals a GGC→GTC at position 2095 and results in a G303V τ mutation (A), and amino acid sequences corresponding to different microtubule-binding domain repeats from human and other mammalian species are shown (B). In B, glycine at position 303 (arrow) is a highly conserved residue present. 1R through 4R indicate 1 through 4 microtubule-binding repeats; MAP, microtubule-associated protein.

Seven family members were tested for the G303V mutation (Figure 1B). Three asymptomatic family members, younger than the average age at onset of disease in this family, are carriers of the mutation (Figure 1B). Two of them were examined and showed normal neurological and neuropsychological examination results, brain magnetic resonance imaging findings, and fluorodopa F 18 uptake.

NEUROPATHOLOGICAL EXAMINATION

There was atrophy of the mesencephalon, pons, striatum, and subthalamic nuclei and depigmentation of the substantia nigra. The lateral ventricles were enlarged, and the frontotemporal cortex showed mild atrophy.

τ Protein accumulation took place in neurons and glia, predominantly in the mesencephalon. In neurons, τ aggregated into neurofibrillary tangles detectable by immunostaining and silver staining. τ Immunostaining was also intense in affected astrocytes. Neuronal loss, mild spongiosis and gliosis, and numerous neurofibrillary tangles were observed in the substantia nigra (**Figure 3**A). No Lewy bodies were seen. Similar lesions were present in the red nucleus, locus coeruleus (Figure 3B), nuclei of the third (Figure 3C and D) and fifth cranial nerves, and nucleus ambiguous. Globose and flame-shaped neurofibrillary tangles were present in these nuclei but not in the nuclei of the 4th and 12th cranial nerves. In addition to tangles, "grumous-degenerative" neurons were observed in the superior colliculus.

Atrophy and neuronal loss were also severe in the globus pallidus and subthalamic nucleus, mild in the hippocampus and parahippocampal gyrus, and moderate in the striatum. In all of these regions, τ accumulation was shown in neurons and glia (Figure 3E). Mild frontal and temporal atrophy was observed with neuronal loss, gliosis, and microvacuolation surrounded by reactive astrocytes and a few τ-positive cells. No significant atrophy was observed in the parietal and occipital cortices, but gliosis and a few τ protein–positive cells were present.

In the dentate nucleus, neurons appeared achromatic, accumulated swollen eosinophilic material, and showed grumous degeneration with accumulation of τ protein (Figure 3F). The lesions did not affect cerebellar white matter or the cerebellar cortex.

MOLECULAR ANALYSIS

Single-stranded conformational polymorphism analysis of τ exon 10 in the proband revealed a DNA band with an abnormal electrophoretic pattern. Direct sequencing analysis identified a mutation that consists of a G→T transversion at position 2095 of the complementary DNA (**Figure 4**A). This mutation changes the glycine residue at position 303 of the protein to valine (G303V). The proband and 3 asymptomatic young relatives were heterozygotes for the mutation (Figure 1B). The deceased and clinically affected members of this family were obligate carriers of the mutation. The mutation was not found in 194 unrelated control chromosomes. The absolute conservation of glycine at position 303 in all human τ repeats, in *MAP2* and *MAP4* (microtubule-associated proteins), and in the second repeat of τ from different species (Figure 4B) and the absence of this mutation in 194 unrelated control chromosomes suggest that G303V is a mutation and not a polymorphism.

τ PROTEIN ISOFORMS IN THE PROBAND BRAIN

The patterns of bands of total τ protein, immunoreactive to antibody 7.51 (**Figure 5**A), and the fraction immunoreactive to PHF-1 (Figure 5B) indicate that the proteins with higher electrophoretic mobility present in the Alzheimer disease–affected brain are absent in the proband. PHF-1 immunoreactivity in Sarkosyl-insoluble pro-

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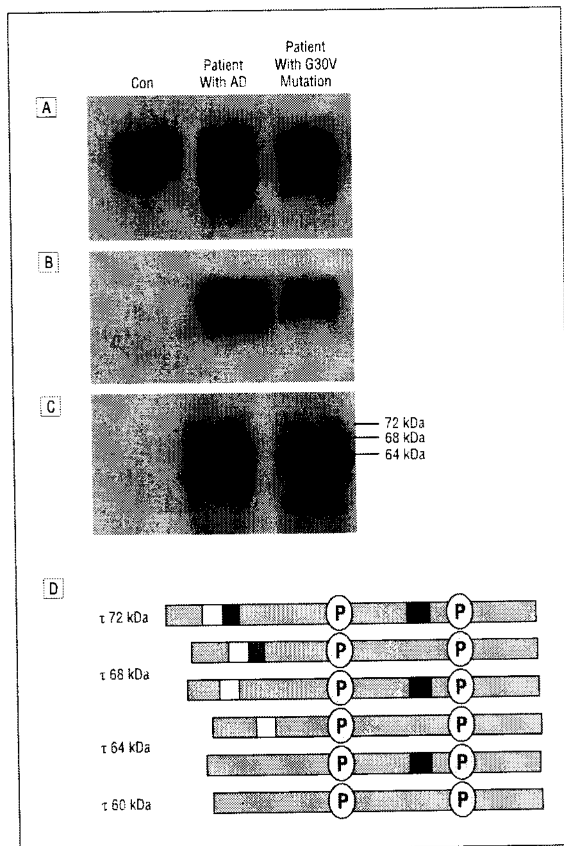

Figure 5. Analysis of τ from a proband brain sample (with the G303V mutation), a matched asymptomatic control subject (Con), and a patient with Alzheimer disease (AD), using τ 7.51 and PHF-1 antibodies. A, Western blot analysis of total extract protein samples from the frontal cortex of all 3 subjects. The blot was immunostained with 7.51 antibody, which recognizes an unphosphorylated epitope of the τ molecule. B, A PHF-1 immunoblot of all 3 subjects. Antibody PHF-1 recognizes hyperphosphorylated τ isoforms in samples from the patient carrying the G303V mutation and from a patient with AD. These bands were completely absent in the brain of the Con. C, Sarcosyl-insoluble extracts from all 3 subjects, immunoblotted with PHF-1 antibody. The presence of proteins with electrophoretic mobility of 72, 68, and 64 kDa is indicated. D, Pattern of phosphoisoforms for tauopathies, like AD and frontotemporal dementia with parkinsonism linked to chromosome 17.[13] P indicates τ phosphorylation.

tein fractions from the proband showed 2 proteins with slower electrophoretic mobility (68 and 64 kDa) (Figure 5C) and, in a smaller amount, a protein of 72 kDa. The 60-kDa protein, corresponding to the smallest 3R τ isoform and lacking exons 2 and 3, is greatly reduced. Only the 72-, 68-, and 64-kDa proteins should contain 4R τ-phosphorylated isoforms (Figure 5D). No reaction was found with PHF-1 for total and particulate control samples (Figure 5B and C).

Figure 6A shows the presence of antibody 7.51 reacting with total τ in the temporal cortex, striatum, and thalamus of the G303V-affected patient. Figure 6B shows PHF-1 immunoreactivity in every brain sample indicated in Figure 6A. Much phosphorylated τ was found in the striatum, a region severely affected. Immunoreactivity to the 7.51 antibody indicates more τ in the striatum of G303V-affected samples than in the striatum of Alzheimer disease–affected samples (Figure 6C and D).

REVERSE TRANSCRIPTION–PCR ANALYSIS OF 4R AND 3R τ ISOFORMS IN THE PATIENT BRAIN

We analyzed the expression of τ isoforms containing or lacking exon 10 by reverse transcription–PCR analysis of messenger RNA samples from the striatum. A proband sample showed an increased proportion of τ complementary DNA containing exon 10 compared with the other samples (**Figure 7**). Amplified control DNA samples containing, or lacking, exon 10 (4R and 3R) are indicated.

COMMENT

Herein, we describe a family with early-onset autosomal dominant PSP due to a novel mutation in the τ protein, G303V. The pathogenesis of this disease seems related to the overexpression of the 4R τ isoform and its hyperphosphorylation. These findings result in a pattern of proteins with relative mobility of 68 and 64 kDa and, in a small amount, of a 72-kDa protein.

In Alzheimer disease and other FTDP-17 cases, an additional band around 60 kDa is present. These 72-, 68-, 64-, and 60-kDa protein bands may correspond to the 6 different central nervous system τ-phosphorylated isoforms,[13,14] but only the 72-, 68-, and 64-kDa proteins contain 4R τ (Figure 5D). Thus, the G303V mutation results in 4R τ, as in other PSP cases.

Mutations of the τ gene are excluded in most patients with PSP,[15,16] but PSP is on linkage disequilibrium for certain polymorphisms and haplotypes of the τ gene. Moreover, patients with mutations in the τ gene may develop atypical PSP features.[7,17]

Around 100 families with mutations in the τ gene have been described. Most of these patients are characterized by either frontal dementia with apathy, desinhibition, hyperorality, or hypersexuality or by atypical, atremoric parkinsonism, axial dystonia, and poor response to dopaminomimetic agents. Typical PSP and FTDP-17 patients are easy to distinguish because they first develop early gate disturbance and prominent brainstem and basal ganglia signs, while the latter usually develop early cognitive deficits or atypical parkinsonian syndromes, while gaze palsy and gait disorders appear late. Atypical cases of both disorders are difficult to differentiate because the clinical phenotypes are variable.[18]

The patients described herein had clinicopathological features of PSP with early age at onset. Supranuclear gaze palsy and gait disorders began early, and dementia appeared late or was questionable. The reduced fluorodopa F 18 uptake was typical of PSP.[19] However, binding of raclopride C 11 was normal and did not suggest dropout of intrinsic striatal neurons. Normal raclopride binding has also been described in some patients with FTDP-17.

The novel G303V mutation of τ seems responsible for this familial disease for the following reasons. (1) It cosegregates with the disease, because it was present in the proband and the other 2 affected members, who were obligate carriers. The 3 asymptomatic gene carriers of the

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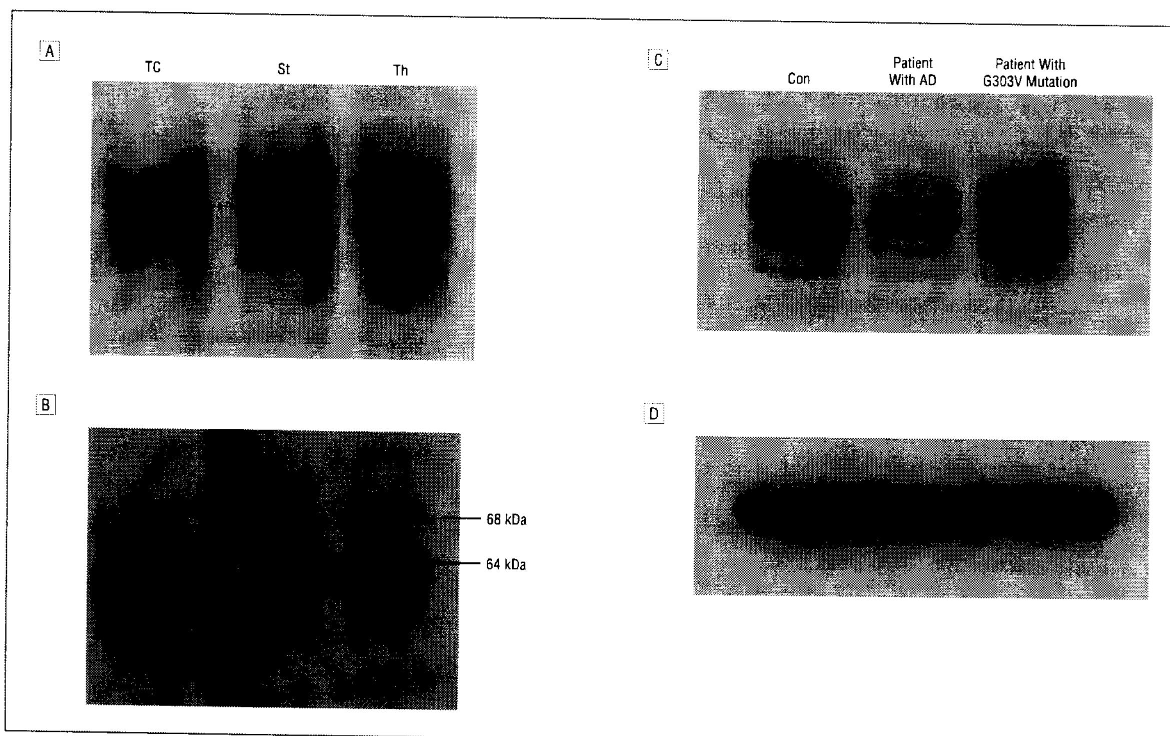

Figure 6. Presence of phosphorylated τ isoforms in the proband's brain. A, Samples from the temporal cortex (TC), striatum (St), and thalamus (Th) from the patient carrying the G303V mutation. A Western blot analysis was performed with the 7.51 antibody. B, A PHF-1 immunoblot of total τ from the TC, St, and Th proband brain regions. C, Analysis of τ protein in St samples from a control subject (Con), a patient with Alzheimer disease (AD), and the proband with the G303V mutation. D, The same St samples as in part C immunoblotted with a β-tubulin antibody.

mutation are younger than the mean age at onset for developing symptoms and may become involved in the future. (2) The mutation does not occur in the healthy population. (3) It takes place in a highly conserved codon of τ protein in several mammal species. (4) The mutation may produce a change in the function of τ.

τ Mutations interfere with protein function by modifying the splicing of exon 10, which alters the normal proportion between the 4R and 3R τ isoforms, and the degree of phosphorylation, which makes τ less efficient in its binding to tubulin and favors its self-assembly.

The mutation G272V, described in an FTDP-17–affected patient,[20] changes the same amino acids in the same motif (PGGG) as the G303V mutation. However, the consequences of both mutations are different. For the G272V mutation, τ was hyperphosphorylated, but τ self-assembly in vitro was modest[21] and the 4R/3R τ ratio was unchanged. For the G303V mutation, τ aggregates were present and the τ 4R/3R ratio was increased, typical features of PSP cases. Thus, other pathogenic mechanisms of action of the G303V mutation should be further investigated.

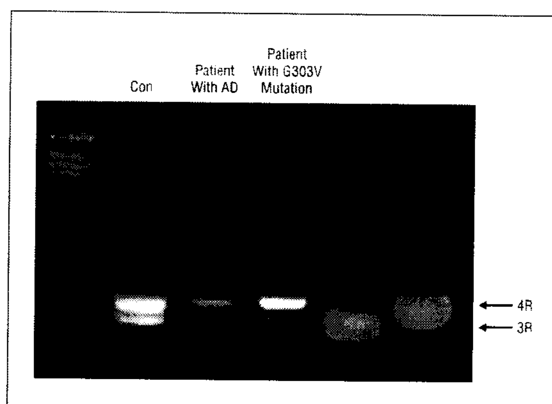

Figure 7. Expression of τ messenger RNA isoforms containing or lacking exon 10 (τ with 4 or 3 microtubule-binding repeats [4R or 3R, respectively]). Reverse transcription–polymerase chain reaction analysis of messenger RNA samples isolated from the striatum of a control subject without dementia (Con), a patient with Alzheimer disease (AD), and the patient with the G303V mutation. The mobilities of τ complementary DNA containing (4R) or lacking (3R) exon 3 are also indicated.

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