ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Автозомно доминантна Паркинсонова болест с късно начало
Определение на заболяването
Автозомнодоминантна Паркинсонова болест с късно начало е във форма на Паркинсонова болест, характеризираща се с начало след 50 години, тремор в покой, нарушения в походката с падания, брадикинезия, ригидност и болезнени крампи. Пациентите са обикновено с нисък риск за развитие на немоторни симптоми, дистония, дискинезии и леводопа-индуцирани дискинезии.
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G20
Код на заболяването по Orpha code
ORPHA411602
Епидемиологични данни за заболяването в Република България
Точната заболеваемост и болестност са неизвестни. Предполага се сходна на останалите страни в Европа.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Milanov I, Kmetska K, Karakolev B, Nedialkov E. Prevalence of Parkinson's disease in Bulgaria. Neuroepidemiology. 2001;20(3):212-4. 2. Farlow J., Pankratz ND, Wojcieszek J., Foroud T. Parkinson Disease Overview GeneReviews® Last Update: February 27, 2014. 3. Paisan-Ruiz C. Orphanet, 2015.
Епидемиологични данни за заболяването в Европейския съюз
Точната заболеваемост и болестност са неизвестни. Превалирането на ПБ се оценява на около 1% от населението над 60 години в общата популация на Европа, като само при 10% от пациентите с ПБ се наблюдава наследственост.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Farlow J., Pankratz ND, Wojcieszek J., Foroud T. Parkinson Disease Overview GeneReviews® Last Update: February 27, 2014. 2. Paisan-Ruiz C. Orphanet, 2015.
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): Автозомно-доминантно унаследявана Паркинсонова болест (съгласно Националния консенсус за диагноза и лечение на Паркинсонова болест) с генетично верифицирана PARK1 (хетерозиготна мутация в alpha-synuclein гена (SNCA) на хромозома 4q22), PARK4 (хетерозиготна трипликация на alpha-synuclein гена (SNCA) на хромозома 4q22), PARK8 (хетерозиготна мутация в гена кодиращ dardarin (LRRK2)), PARK11 (хетерозиготна мутация в GIGYF2 гена на хромозома 2q37), PARK17 (хетерозиготна мутация в VPS35 гена на хромозома 16q13), PARK18 (хетерозиготна мутация в EIF4G1 гена на хромозома 3q27) и PARK21.

Признаците и симптомите на заболяването: В клиничната картина треморът в покой се наблюдава в 70% от случаите, нарушенията в походката с падания при 20%, брадикинезия при 20% и ригидност/болезнени крампи при 8%. В сравнение с ПБ с ранно начало при наследствената ПБ с късно начало е налице повишен риск за развитие на нарушения в походката с падания, но от друга страна е по-нисък рискът за развитието на дистония, дискинезии и леводопа-индуцирани дискинезии. Пациентите с наследствена ПБ с късно начало са също по-засегнати от тежка диплопия, когнитивни нарушения, гастроинтестинални и уринарни нарушения. Тези пациенти обаче съобщават за по-редки други немоторни симптоми, като депресия, халюцинации, поведенчески нарушения (ажитираност или impulse control disorder), деменция и апатия.

Етиологията и патогенезата: Точната етиология на Автозомно-доминантно унаследяваната Паркинсонова болест е все още неизвестна, но мутации в гените SNCA (4q21.3-q22), LRRK2 (12q12) и VPS35 (16q12) се приема, че са обвързани с патологичния процес. Lotharius и Brundin (2002) предполагат възможна роля на SNCA в рециклирането на везикулите чрез регулиране на фосфолипаза D2 и чрез неговата мастна киселина-свързващи свойства. Авторите предполагат, че нарушеното натрупване на невротрансмитер, което се дължи на SNCA мутации, може да доведе до цитоплазмено натрупване на допамин с последващ разпад на този лабилен невротрансмитер в цитоплазмата и повишаване на оксидативния стрес и метаболитната дисфункция в субстанция нигра.

При PARK4 с SNCA трипликация се предполага, че увеличената доза на SNCA е причина за ПБ. Gehrke и колеги (2010) установяват, че LRRK2 взаимодейства с microRNA (miRNA) път за регулация на белтъчната синтеза, което вероятно е свързано с PARK8. Chartier-Harlin и колеги (2011) предполагат при PARK18, че мутациите възпрепятстват способностите на клетките за бърз и динамичен отговор на стрес, вероятно чрез промяна в транслацията на съществуващата mRNAs, което е съществено за клетъчното оцеляване.

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

1. Национален консенсус за диагноза и лечение на Паркинсонова болест, Двигателни заболявания, 2013, 1,1.
2. Golbe, L. I., Di Iorio, G., Bonavita, V., Miller, D. C., Duvoisin, R. C. A large kindred with autosomal dominant Parkinson's disease. Ann. Neurol. 27: 276-282, 1990.
3. Golbe, L. I., Di Iorio, G., Sanges, G., Lazzarini, A. M., La Sala, S., Bonavita, V., Duvoisin, R. C. Clinical genetic analysis of Parkinson's disease in the Contursi kindred. Ann. Neurol. 40: 767-775, 1996.
4. Spira, P. J., Sharpe, D. M., Halliday, G., Cavanagh, J., Nicholson, G. A. Clinical and

pathological features of a Parkinsonian syndrome in a family with an ala53-to-thr alpha-synuclein mutation. Ann. Neurol. 49: 313-319, 2001.

5. Puschmann, A., Ross, O. A., Vilarino-Guell, C., Lincoln, S. J., Kachergus, J. M., Cobb, S. A., Lindquist, S. G., Nielsen, J. E., Wszolek, Z. K., Farrer, M., Widner, H., van Westen, D., Hagerstrom, D., Markopoulou, K., Chase, B. A., Nilsson, K., Reimer, J., Nilsson, C. A Swedish family with de novo alpha-synuclein A53T mutation: evidence for early cortical dysfunction. Parkinson. Relat. Disord. 15: 627-632, 2009.
6. Golbe, L. I., Lazzarini, A. M., Schwarz, K. O., Mark, M. H., Dickson, D. W., Duvoisin, R. C. Autosomal dominant parkinsonism with benign course and typical Lewy-body pathology. Neurology 43: 2222-2227, 1993.
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10. Farrer, M., Kachergus, J., Forno, L., Lincoln, S., Wang, D.-S., Hulihan, M., Maraganore, D., Gwinn-Hardy, K., Wszolek, Z., Dickson, D., Langston, J. W. Comparison of kindreds with parkinsonism and alpha-synuclein genomic multiplications. Ann. Neurol. 55: 174-179, 2004.
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12. Waters, C. H., Miller, C. A. Autosomal dominant Lewy body parkinsonism in a four-generation family. Ann. Neurol. 35: 59-64, 1994.
13. Singleton, A. B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R., Lincoln, S., Crawley, A., and 10 others. Alpha-synuclein locus triplication causes Parkinson's disease. Science 302: 841 only, 2003.
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linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum. Molec. Genet. 12: 2599-2608, 2003.
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20. Vilarino-Guell, C., Rajput, A., Milnerwood, A. J., Shah, B., Szu-Tu, C., Trinh, J., Yu, I., Encarnacion, M., Munsie, L. N., Tapia, L., Gustavsson, E. K., Chou, P., and 28 others. DNAJC13 mutations in Parkinson disease. Hum. Molec. Genet. 23: 1794-1801, 2014.

Алгоритми за диагностициране на заболяването

Алгоритми за диагностициране на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест, а на когнитивните нарушения съгласно Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

Анамнезата: В клиничната картина треморът в покой се наблюдава в 70% от случаите, нарушенията в походката с падания при 20%, брадикинезия при 20% и ригидност/болезнени крампи при 8%. В сравнение с ПБ с ранно начало при наследствената ПБ с късно начало е налице повишен риск за развитие на нарушения в походката с падания, но от друга страна е по-нисък рискът за развитието на дистония, дискинезии и леводопа-индуцирани дискинезии. Пациентите с наследствена ПБ с късно начало са също по-засегнати от тежка диплопия, когнитивни нарушения, гастроинтестинални и уринарни нарушения. Тези пациенти обаче съобщават за по-редки други немоторни симптоми, като депресия, халюцинации, поведенчески нарушения (ажитираност или impulse control disorder), деменция и апатия.

Диференциалната диагноза на заболяването: атипичен паркинсонизъм, вторичен паркинсонизъм

Лабораторни, образни и хистологични изследвания: PET/SPECT изследванията показват намалено натрупване на допаминовите транспортери в базалните ганглии.

Генетични изследвания и медико-генетично консултиране: LOPD е с автозомно-доминантно унаследяване. Генетична консултация трябва да се предлага на засегнатите семейства, като биват информирани, че има 50% вероятност за поколението да наследи мутацията, причиняваща заболяването и следователно да развият заболяването.

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

1. Националния консенсус за диагноза и лечение на Паркинсонова болест, Двигателни заболявания, 2013, 1,1.
2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
3. Golbe, L. I., Di Iorio, G., Bonavita, V., Miller, D. C., Duvoisin, R. C. A large kindred with autosomal dominant Parkinson's disease. Ann. Neurol. 27: 276-282, 1990.
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kindred. Ann. Neurol. 40: 767-775, 1996.
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9. Muenter, M. D., Forno, L. S., Hornykiewicz, O., Kish, S. J., Maraganore, D. M., Caselli, R. J., Okazaki, H., Howard, F. M., Jr., Snow, B. J., Calne, D. B. Hereditary form of parkinsonism-dementia. Ann. Neurol. 43: 768-781, 1998.
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17. Vilarino-Guell, C., Rajput, A., Milnerwood, A. J., Shah, B., Szu-Tu, C., Trinh, J., Yu, I., Encarnacion, M., Munsie, L. N., Tapia, L., Gustavsson, E. K., Chou, P., and

28 others. DNAJC13 mutations in Parkinson disease. Hum. Molec. Genet. 23: 1794-1801, 2014.
Алгоритми за лечение на заболяването
Алгоритми за лечение на заболяването (Терапевтичните подходи към заболяването, в това число консервативни и оперативни, техните предимства, рискове и очаквана ефективност; Препоръчителен диетичен режим и физическа активност и др.: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и 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 диагноза и лечение на Паркинсонова болест, Двигателни заболявания, 2013, 1,1. 2. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
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 диагностика и лечение на Паркинсоновата болест и 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 диагноза и лечение на Паркинсонова болест, Двигателни заболявания, 2013, 1,1. 2. 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 диагностика и лечение на Паркинсоновата болест и 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 диагноза и лечение на Паркинсонова болест, Двигателни заболявания, 2013, 1,1. 2. 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: Първична, вторична и третична превенция: LOPD е с автозомно-доминантно унаследяване. Генетична консултация трябва да се предлага на засегнатите семейства, като биват информирани, че има 50% вероятност за поколението да наследи мутацията причиняваща заболяването и следователно да развият заболяването.
В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Farlow J., Pankratz ND, Wojcieszek J., Foroud T. Parkinson Disease Overview GeneReviews® Last Update: February 27, 2014.
2. Paisan-Ruiz C. Orphanet, 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)

Моля, опишете опита в Република България с конкретни пациенти със съответното рядко заболяване. Моля, подкрепете информацията с данни от научни публикации от последните пет години. Библиографската информация следва да бъде оформена в стил Ванкувър.

Опитът на кандидатстващия експертен център под ръководството на чл. кор. проф.Трайков за диагноза и лечение на редки заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, 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 реферирал център за заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, 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 центъра са осъществени няколко дисертации в областта: 1. Когнитивни нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Мария Петрова, 2010 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Лонгитудинално проследяване на когнитивните нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Явор Желев, 2012 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. 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). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, протичащи с паркинсонизъм с и без когнитивен дефицит 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, протичащи с моторни и когнитивни нарушения, 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.

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ORIGINAL CONTRIBUTION

α-Synuclein Gene Rearrangements in Dominantly Inherited Parkinsonism

Frequency, Phenotype, and Mechanisms

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Objective: Genomic multiplications of the α-synuclein gene (*SNCA*) cause autosomal dominant Parkinson disease (ADPD). The aim of this study was to assess the frequency and phenotype of *SNCA* rearrangements in a large series of families with typical or atypical AD parkinsonism.

Design: Patients were screened by the exon dosage of the *SNCA* gene. The genotype of patients and relatives carrying *SNCA* rearrangements, the size of the multiplied regions, and the centromeric and telomeric breakpoints were determined by microsatellite dosage and 250K Affymetrix Single Polymorphism Nucleotide microarrays (Affymetrix, Santa Clara, California).

Subjects: Index cases and, whenever appropriate, relatives of 286 mainly European families with ADPD were screened.

Results: Four of 264 families (1.5%) with typical ADPD carried duplications and 1 of 22 families (4.5%) with atypical AD parkinsonism carried a triplication of *SNCA*. Genotyping and dosage analyses showed that the multiplied regions were variable in size (0.42-5.29 megabase pairs), suggesting that *SNCA* multiplications occurred independently. Phenotype analyses showed that the severity of the disease correlated with the *SNCA* copy number, but not with the minimal number of multiplied genes (1 to 33). Haplotype analysis of polymorphic markers suggested that multiplication of the *SNCA* gene occurred by both interchromosomal and intrachromosomal rearrangement.

Conclusions: Our results suggest that *SNCA* rearrangements may be more frequent than point mutations in ADPD. Furthermore, our results indicate that the phenotype associated with *SNCA* multiplications correlates with the number of copies of the gene and provides the first insight into the mechanisms underlying *SNCA* multiplication.

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PARKINSON DISEASE (PD) (OMIM 168600) is a frequent progressive neurodegenerative disorder. Symptoms are caused mainly by loss of dopaminergic neurons in the substantia nigra. However, the mechanisms by which these neurons degenerate remain unknown. The study of familial forms of the disease compatible with mendelian inheritance and accounting for less than 10% of PD cases[1] has led to the identification of 11 genes and/or loci implicated in the pathology.[2] Polymeropoulos et al[3] identified the first mutation causing PD (p.A53T) in the α-synuclein gene (*SNCA*; OMIM 163890) in a large Italian American family with autosomal dominant parkinsonism. After intensive screening of the *SNCA* gene in patients with PD of different origins, only 2 additional point mutations have been identified (p.A30P[4] and p.E46K[5]), suggesting that point mutations in *SNCA* are a rare cause of PD. In contrast, different duplications and triplications of the whole *SNCA* gene are found in families with autosomal dominant forms of the disease.[6-11] More recently, *SNCA* duplications were also identified in rare, apparently sporadic cases.[12,13] The severity of the phenotype, ranging from typical PD to dementia with Lewy bodies (*DLB*); (OMIM 127750) and PD dementia, and the brain structures affected have been shown to increase with the number of copies of the gene. Most *SNCA* duplication carriers (3 copies of the gene) have typical late-onset parkinsonism,[6-9] whereas those with triplications (4 copies of the gene) have earlier-onset and atypical parkinsonism.[10,11] However, owing to the limited number of studies, the prevalence and mechanisms leading to these rearrangements have not been precisely determined.

The aim of this study was to assess the frequency of *SNCA* rearrangements in a large series of families with typical or atypi-

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Group Information: The members of the French Parkinson's Disease Genetics Study Group are listed at the end of this article.

cal parkinsonism compatible with autosomal dominant inheritance and to determine the mechanism by which they occur.

METHODS

PATIENTS

In this study, 345 patients with parkinsonism from 286 families (including 1[4] and 119[8] already published) compatible with autosomal inheritance were examined by a member of the French Parkinson's Disease Genetics Study Group. A total of 210 families had at least 1 affected parent-child pair, and 76 families had at least 2 affected relatives in 2 different generations (uncle-aunt and/or nephew-niece pairs). In addition, 37 of the 76 index cases had an affected sibling. Diagnostic criteria for definite PD were the presence of at least 3 of the following cardinal signs: resting tremor, bradykinesia, rigidity, and good (>30%) response to levodopa therapy. In 264 of the families, exclusion criteria such as ophthalmoplegia, dementia, and dysautonomia early in the course of the disease, as well as apraxia, cerebellar, and pyramidal syndromes were absent. The clinical features of the 3 families carrying duplications (FPD-131,[5] FPD-321, and FPD-410[8]) have already been published. In addition, we screened 22 index cases with atypical parkinsonism. These patients had 1 or more atypical symptoms including pyramidal signs (n=8), early dementia (n=7), myoclonus (n=2), apraxia (n=2), supranuclear ophthalmoplegia (n=2), hypoventilation (n=1), axonal neuropathy (n=1), dysautonomia (n=1), no response to levodopa treatment (n=2), cerebellar ataxia (n=1), and subacute onset (n=1).

The mean (SD) age at onset of disease in index patients (140 women; 146 men) was 48.6 (13.2) years (range, 8-86 years) and at examination was 57.3 (13.0) years (range, 25-88 years). The 286 families were mostly of European descent (n=259), including families from France (n=230), Italy (n=11), Spain (n=4), Germany (n=3), the United Kingdom (n=3), Portugal (n=3), the Netherlands (n=1), Ireland (n=1), Greece (n=1), and Eastern Europe (n=2). There were also patients from North Africa (n=15), South (n=2) and North America (n=1), the West Indies (n=5), Turkey (n=1), Asia (n=1), and the Middle East (n=2).

Local ethics committees approved the study and written informed consent was obtained from all participants. Peripheral blood was collected from each patient; genomic DNA was extracted from leukocytes according to standard procedures and was used for subsequent molecular analyses.

MOLECULAR ANALYSES

Detection of *SNCA* Rearrangements

Dosage of the *SNCA* gene was performed by semiquantitative multiplex polymerase chain reaction (PCR), as previously described.[8] Briefly, exons 3 and 4 of this gene were coamplified with 2 internal controls: a 236–base pair sequence of a reference gene, transthyretin (OMIM 176300), and the exon 4 of the parkin gene (OMIM 602544). Two diploid subjects were used as negative controls and a patient with a known heterozygous deletion of parkin exon 4 as positive control. The PCR products were quantified using GeneMapper 3.5 software (Applied Biosystems, Foster City, California) on an ABI 3730 genetic analyzer (Applied Biosystems). The number of copies of the gene was determined from the ratios of mutant to control alleles compared with ratios obtained from DNA of the control subjects. Ratios between 0.8 and 1.2 indicated a normal individual; between 1.3 and 1.7, a heterozygous duplication; and between 1.8 and 2.2, a triplication or homozygous duplication. Multiplied regions were further validated with intronic and flanking microsatellite markers in available members of positive families as previously reported[8]; probands were tested for the *LRRK2* G2019S mutation. All tests were performed at least twice.

Refinement of the Multiplied Regions

To determine the extent of the multiplied region in each positive family, we analyzed 23 existing and 13 newly developed quantitative microsatellite markers spanning approximately 8 megabase pairs (Mb) within the 4q21.3 to 4q22.3 genomic region (primer sequences available on the Genome Database human genome database Web site; http://www.gdb.org).

To pinpoint the breakpoint ends of the multiplied regions spanning *SNCA*, Affymetrix GeneChip Human Mapping 250K Nsp arrays genotyping and single-nucleotide polymorphism (SNP) dosage analysis were performed according to the manufacturer's protocol (Affymetrix, Santa Clara, California). Affymetrix GCOS v1.4 software was used to obtain raw microarray feature intensities (Raw microarray feature intensities [RAS] scores; raw data available on request). The RAS scores were processed using Affymetrix GTYPE v4.0 software to derive SNP genotypes. Copy number estimates were obtained using the program dChip.[14-16] Raw copy number estimates were then smoothed using a Hidden Markov Model approach that treats copy number along the chromosome as a Markov process with a very low probability of switching between different copy number states.

The minimal size of the multiplied regions was defined by the last informative multiplied microsatellite or SNP marker and the maximal size extended up to the first informative but not multiplied marker on each boundary of the *SNCA* gene.

Bioinformatics Analyses

Information available from the Ensembl Genome Browser (http://www.ensembl.org), the University of California Santa Cruz Genome Browser (http://genome.ucsc.edu/), the National Center for Biotechnology Information Web site (http://www.ncbi.nlm.nih.gov/), and the Genome Database Web site (http://www.gdb.org) was used to design PCR primers and to identify known and predicted genes in the multiplied regions. Repeat elements and low-copy repeats at the centromeric and telomeric boundaries of each multiplied region were searched for using the RepeatMasker (http://www.repeatmasker.org/) and BLASTN (http://www.ncbi.nlm.nih.gov/blast/) programs. For this purpose, genomic sequences of interspersed sequence repeats that had been filtered out were compared with the complete human sequence genome draft.

Haplotype Analysis

To identify alleles shared among and within families and to further analyze the mechanism of the rearrangement in each family, haplotypes for all available members of families with *SNCA* gene multiplications were constructed manually, allowing a minimum number of recombinations. We used 7 microsatellite markers (NACP-REP1 and 6 newly developed markers) located within the shared *SNCA*-containing region. Flanking markers NACP-REP1 and D4S3460 are located approximately 9 and 10 kilobase pairs (Kb) from the 5′ and 3′ ends of the *SNCA* gene, respectively. The D4S3455 and D4S3456 markers are located in introns 1 and 2, respectively, with D4S3457, D4S3458, and D4S3459 in intron 3 of the *SNCA* gene.

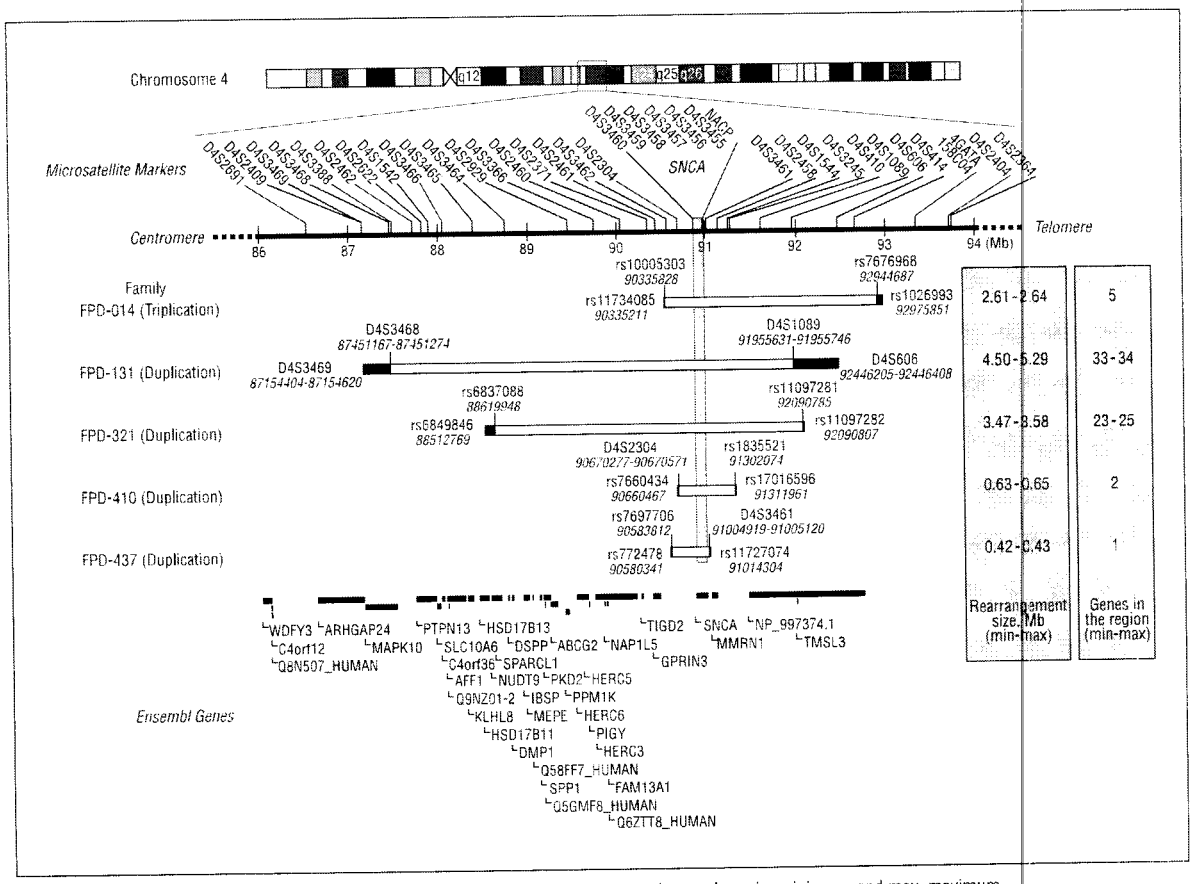

Figure 1. Breakpoint analysis of 5 families with *SNCA* multiplications. Mb indicates megabase pairs; min, minimum; and max, maximum.

Statistical Analysis

Comparisons between patient groups were made with the χ^2 test or the Fisher exact test when appropriate for qualitative variables and analyses of variance for quantitative variables using SPSS Software (SPSS Inc, Chicago, Illinois).

RESULTS

DETECTION AND REFINEMENT OF THE MULTIPLIED REGION CONTAINING *SNCA*

Using semiquantitative multiplex PCR to detect exon rearrangements, we found *SNCA* duplications in 4 families and a triplication in 1, but no deletions. The presence of extra copies of the whole *SNCA* gene in these families was confirmed by analyzing 2 flanking and 7 intragenic *SNCA* microsatellite markers. The families were from France (FPD-014, FPD-131, FPD-410, FPD-437) and Italy (FPD-321). All affected members of these families whose DNA was analyzed were heterozygous carriers of an *SNCA* multiplication. Analyses of 36 polymorphic microsatellite markers confirmed these results and allowed estimation of the size of the rearrangements. Refinement of the multiplied regions sizes in 4 of the 5 families was performed by dosage analysis using Affymetrix 250K SNP microarrays. In the remaining family (FPD-131), the duplication was confirmed, but the boundaries could not be refined because no more genomic DNA was available for analysis. The minimal size of the multiplied regions differed among the families and ranged from 0.42 to 4.50 Mb, suggesting that the events occurred independently (**Figure 1**). Family FPD-014 had a triplicated region of 2.61 to 2.64 Mb that contained 4 known genes (*SNCA*, multimerin 1 [*MMRN1*] [OMIM 601456], thymosin-like 3 [*TMSL3*; NM_183049], G protein–regulated inducer of neurite outgrowth 3 [*GPRIN3*] [OMIM 611241]), and a gene encoding the hypothetical protein LOC401145 (NP_997374.1; NM_207491). Members of family FPD-131 had the largest duplication (4.50-5.29 Mb), according to microsatellite dosage, which contained 33 to 34 genes (Figure 1). Several of these genes encode mitogen-activated protein (MAP) kinases or phosphatases; 3 others encode members of the HECT domain and RCC1-like domain family (*HERC1*) of ubiquitin ligases. Five of these genes, the MAP kinase 10 (*MAPK10*) (OMIM 602897), *SNCA*, *MMRN1*, polycystic kidney disease 2 (*PKD2*) (OMIM 173910), and dentin matrix acidic phosphoprotein (*DMP1*; OMIM 600980) genes have been associated with human diseases. The duplicated region in family FPD-321 was the second largest (3.47-3.58 Mb) and contained at least 23 genes. The duplicated region in family FPD-410 (approximately

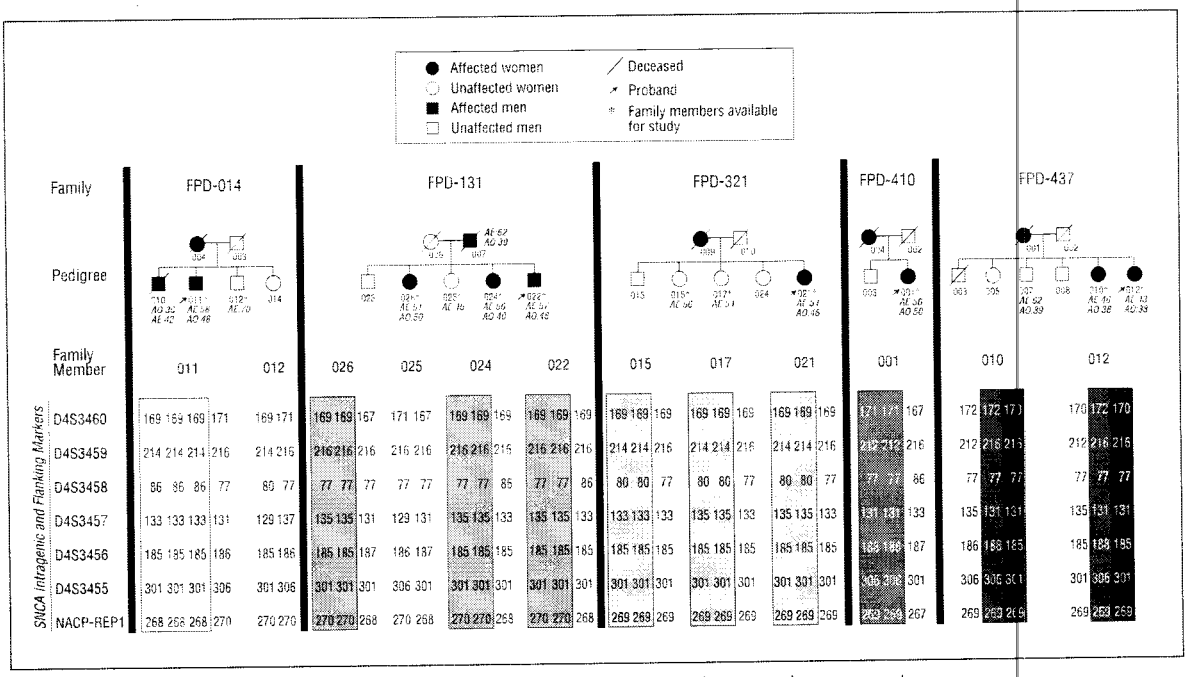

Figure 2. Duplications and triplications of the *SNCA* gene caused by intrachromosomal or interchromosomal rearrangements.

0.63-0.65 Mb) contained *SNCA* and *MMRN1*. The duplication in family FPD-437 (approximately 0.42-0.43 Mb) contained only *SNCA*.

RepeatMasker software revealed a variable number of interspersed repetitive elements, including Alu elements, long interspersed nuclear elements, and long terminal repeats in both the centromeric and telomeric boundaries of the multiplied regions containing *SNCA* in all families except FPD-321 and FPD-014. No low-copy repeats, 10- to 400-Kb DNA blocks with more than 95% identity between copies, were found in the intervals containing the breakpoints.

HAPLOTYPE ANALYSIS

Analysis of 7 microsatellite markers within the *SNCA* multicopy region showed that each family had a different set of alleles, confirming that the mutations were not inherited from a common ancestor (**Figure 2**). In families FPD-131, FPD-321, and FPD-410, all *SNCA* duplication carriers had 2 identical copies of the region (Figure 2). Similarly, the *SNCA* triplication carrier FPD-014-011 had 3 identical copies of the triplicated haplotype plus a haplotype of the region that he shared with his unaffected brother. In contrast, both duplication carriers from family FPD-437 shared a duplicated region containing 2 haplotypes, but the haplotype of the third copy of the region was not shared (Figure 2).

CLINICAL CHARACTERISTICS

All families with duplications had typical PD, whereas the family with a triplication had atypical features. Patients with duplications and triplications of the *SNCA* gene had similar ages at examination, ages at onset, and disease durations (**Table**). In the 4 families with *SNCA* duplications, all patients had typical PD that was similar to the phenotype of patients with typical autosomal dominant PD (ADPD) without *SNCA* rearrangements. Two *SNCA* duplication carriers were still asymptomatic at ages 56 and 54 years (FPD-321-015 and FPD-321-017; Figure 2). By 8 and 10 years of age, respectively, both were older than the age at onset of their affected sister (FPD-321-21) whose disease began at 46 years but had not reached the latest age at onset in patients with duplications in this series, which was 65 years. These observations were consistent with incomplete age-dependent penetrance.

The patients carrying *SNCA* triplications had atypical parkinsonism with rapidly evolving symptoms and severe cognitive impairment. Their response to levodopa treatment was limited at onset or decreased very rapidly after onset. Triplication carriers had shorter disease durations until death than duplication carriers (mean [SD] disease duration, 7.0 [2.6] years; range, 5-10 years; n=3 vs 10.5 [7.2] years; range, 1-23 years; n=3), assuming that patients who could not be analyzed for gene dosage carried the multiplication. Owing to the small number of cases, however, the difference was not statistically significant. Despite shorter disease durations, patients with triplications had more severe dementia (mean [SD] Mini-Mental State Examination score, 15.0 [12.8]; range, 6-24; n=2 vs 27.8 [2.0]; range, 26-30; n=5; $P=.05$), were more severely affected (mean [SD] Unified Parkinson Disease Rating Scale motor score during treatment, 44.5 [2.1]; range, 43-46; n=2 vs 11.7 [6.1]; range, 5-17; n=3; $P=.006$), and had urinary incontinence more frequently (3/3 vs 0/6; $P=.01$) than those with duplications.

Table. Clinical Comparison of Patients With PD With Typical or Atypical Presentations and With *SNCA* Duplications or Triplications[a]

Characteristics	*SNCA* Triplication (n=3, 1 Genotyped) Mean (SD)	Range (Available Data[b])	*SNCA* Duplication (n=9, 7 Genotyped) Mean (SD)	Range (Available Data[b])	*P* Value	Atypical Parkinsonism (n=24) Mean (SD)	Range (Available Data[b])	Typical Parkinsonism (n=313) Mean (SD)	Range (Available Data[b])
Sex, women:men	1:2		7:2		.24	16:18		155:158	
Age, y	55.3 (12.2)	42-66	56.6 (11.8)	43-84	.88	56.8 (15.2)	25-88	58.7 (13.7)	25-90
Age at onset, y	48.3 (12.5)	36-61	46.0 (8.7)	38-65	.72	49.9 (14.3)	18-76	49.9 (13.9)	8-86
Disease duration, y	7.0 (2.6)	5-10	10.5 (7.2)	1-23	.43	6.9 (7.8)	0-33	8.7 (6.9)	0-52
Age at death, y	58.7 (15.6)	42-73 (3)	67.7 (14.4)	57-84 (3)	.50				
Clinical signs at onset									
Micrography, No.	0/3		1/7		>.99	10/41		107/370	
Rest tremor, No.	2/3		2/7		.5	18/43		218/371	
Bradykinesia, No.	2/3		6/7		>.99	21/43		226/372	
Asymmetry, No.	2/3		7/9		>.99	36/42		352/375	
Self-estimated levodopa improvement	36 (22)	17-60	53 (18)	40-60	.43	34 (19)	10-100	66 (19)	30-100
Clinical signs at examination									
Bradykinesia, No.	3/3		9/9			35/44		368/383	
Rigidity, No.	3/3		8/9		>.99	39/43		358/380	
Rest tremor, No.	2/2		6/9		>.99	30/47		295/377	
Urinary incontinence, No.	3/3		0/6		.01	11/40		25/367	
MMSE score[c]	15.0 (12.8)	6-24 (2)	27.8 (2.0)	26-30 (5)	.05	25 (7.0)	6-30 (18)	27.6 (4.0)	5-30 (212)
UPDRS score on[d]	44.5 (2.1)	43-46 (2)	11.7 (6.1)	5-17 (3)	.006	26.2 (16.2)	7-57 (12)	19.2 (13.5)	0-80 (199)
UPDRS score off[d]	51.2 (6.7)	47-56 (2)	42.7 (27.1)	5-86 (6)	.69	34.8 (14.9)	17-56 (10)	35.5 (19.0)	5-96 (130)
Daily dose of levodopa, mg	1000 (283)	800-1200 (2)	450 (397)	50-1100 (5)	.14	387 (364)	0-1200 (19)	577 (336); n=244	0-2300 (244)
Duration of treatment, y	NA		6.6 (3.4)	3-12 (5)		7.1 (6.9)	0.3-24 (19)	6.8 (6)	0.2-29 (304)
Dyskinesia	NA		4/5			7/21		154/325	
Fluctuation	NA		3/5			7/21		175/317	
Dystonia	NA		2/4			6/20		96/315	

Abbreviations: MMSE, Mini-Mental State Examination; NA, not available; PD, Parkinson disease; UPDRS, Unified Parkinson Disease Rating Scale.
[a] Comparisons of means and frequencies were calculated using SPSS software (SPSS Inc, Chicago, Illinois).
[b] Indicates the number of patients with available data for the corresponding feature.
[c] The maximum score for the MMSE is 30 points.
[d] The maximum score for the UPDRS is 180. On and off indicate that the scale was rated with or without treatment with levodopa, respectively.

COMMENT

In addition to PD caused by *SNCA* multiplications, a very limited number of human genetic diseases have been reported to be caused by increased gene dosage, including autism spectrum disorder (OMIM 209850, caused by duplication or deletion of contactin 4 [*CNTN4*] (OMIM 607280]),[17] Charcot-Marie Tooth disease type 1A (OMIM 118220, caused by duplication of the peripheral myelin protein 22 [OMIM 601097] locus),[18] Pelizaeus-Merzbacher disease (OMIM 312080, caused by duplication of proteo-lipid protein 1 [OMIM 300401]),[19] early-onset Alzheimer disease (OMIM 104760.0020, caused by duplication of the amyloid precursor protein [OMIM 104760] locus),[20] and hereditary pancreatitis (OMIM 167800, caused by triplication of the trypsinogen [OMIM 276000] locus).[21] In this study, we found 4 of 264 families (1.5%) with typical ADPD that carried *SNCA* gene duplications. Our results are in accordance with a smaller study in an Asian population[7] in which *SNCA* duplications were found in 2 of 113 families with ADPD (1.8%). Because *SNCA* triplications have been associated with dementia with Lewy bodies and PD dementia, we also screened a series of 22 patients with autosomal dominant atypical parkinsonism and found 1 family who carried an *SNCA* gene triplication (4.5%). In contrast, several other studies failed to identify *SNCA* rearrangements in patients with PD or dementia with Lewy bodies, but the number of familial cases was limited.[22-27] Our results suggest that *SNCA* duplications may be more frequent than point mutations in familial PD.

As anticipated from previous studies, duplications produced typical PD, whereas triplications resulted in an earlier onset and more aggressive disease with features reminiscent of dementia with Lewy bodies, indicating that the increase in the number of copies of *SNCA* has an effect on phenotype.[6-11] However, 2 recent studies reported *SNCA* duplications in patients with either dementia[7] or variable symptoms resembling those of multiple system atrophy.[6] The absence of dementia in our patients with *SNCA* duplications might be due to shorter disease durations or to ages at onset that were earlier than those previously reported (mean [SD], 46.0 [8.7] vs 52.0 [13.3] years).[6,7,9] Conversely, the age at onset in our patients with an *SNCA* triplication was later (48.3 [12.5] years) than that of the Iowan (36.0 [10.5] years)[11] or Swedish American (31 years) patients.[6,10] Comparison of patients carrying duplications and triplications of the *SNCA* gene showed no statistically significant differences in age at examination, age at onset, or disease duration. However, patients with triplications had shorter disease durations until death than duplication carriers. They had more severe dementia, were more severely affected, and had more frequent urinary incontinence than those with

duplications, underlining the dosage effect of *SNCA* multiplications.

The size of the rearrangements estimated by dosage of microsatellite markers was confirmed and refined by Affymetrix 250K SNP microarrays in all but one family who could not be analyzed because no more genomic DNA was available. Interestingly, our study shows that the size of the duplicated region and the number of genes it contains has no effect on phenotype. There are no additional signs or greater severity in family FPD-131 with at least 33 more duplicated genes than in families in which *SNCA* alone (FPD-437) or *SNCA* and *MMRN1* (FPD-410) were duplicated. Nishioka et al[7] speculated that *MMRN1* overexpression may contribute to the dementia phenotype because one demented patient carried a genomic duplication containing both *SNCA* and *MMRN1* in their entirety, whereas the duplicated region in the patient without dementia contained all of *SNCA* but only part of *MMRN1*. Our results do not confirm this hypothesis because the patients carrying multiplications of both *SNCA* and *MMRN1* in our series did not have dementia. In addition, the *MMRN1* gene is not expressed in the brain.

Penetrance of *SNCA* multiplications is age dependent and might also be reduced, as suggested by previous studies.[7,12] However, the genetic basis of this age-at-onset variability is not known. It is probably not related to the size of the multiplication because the 2 unaffected carriers belong to the family with the second largest duplication. We have also excluded the *LRRK2* G2019S mutation, which was absent in all of the families with *SNCA* multiplication, as a potential modifier.

The mutations found in the 5 families have different sizes, and the haplotypes in the rearranged region are not the same, indicating that all 5 mutations occurred independently. Furthermore, our haplotype analysis suggests that different mechanisms are involved in the generation of *SNCA* multiplications. For 3 duplications and the triplication, a single sequence was multiplied and transmitted in each family. This suggests an unequal intrachromosomal rearrangement after recombination between sister chromatids. In the 2 patients of family FPD-437, however, the microsatellite alleles on the 2 copies of the duplicated region were different, suggesting that the duplication resulted from a nonallelic homologous recombination between 2 homologous chromosomes. This mechanism is frequent in genomic disorders and is usually the consequence of nonallelic homologous recombination between low-copy repeats that flank unique genomic segments.[28] However, in our study, bioinformatics analyses of all of the multiplied regions containing *SNCA* revealed no large flanking low-copy repeats, but rather numerous repetitive elements interspersed at the breakpoints. Interspersed repetitive elements at breakpoints have been reported elsewhere and their presence has been postulated to be a frequent cause of deletions and duplications by nonallelic homologous recombination.[29-31] Interestingly, the most comprehensive study of genome-wide human segmental duplications found an enrichment of Alu repeats near or within breakpoints.[32]

Our study indicates that the phenotype associated with *SNCA* multiplications correlates with the number of copies of the *SNCA* gene, but not with the number of copies of other genes in the multiplied region, and provides the first insight into the mechanisms underlying *SNCA* multiplication. Because disorders resulting from genome rearrangements are thought to occur with equal frequency in all populations,[33] *SNCA* multiplications could account for approximately 2% of all ADPD cases.

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A Swedish family with *de novo* α-synuclein A53T mutation: Evidence for early cortical dysfunction

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ABSTRACT

A *de novo* α-synuclein A53T (p.Ala53 Th; c.209G > A) mutation has been identified in a Swedish family with autosomal dominant Parkinson's disease (PD). Two affected individuals had early-onset (before 31 and 40 years), severe levodopa-responsive PD with prominent dysphasia, dysarthria, and cognitive decline. Longitudinal clinical follow-up, EEG, SPECT and CSF biomarker examinations suggested an underlying encephalopathy with cortical involvement. The mutated allele (c.209A) was present within a haplotype different from that shared among mutation carriers in the Italian (Contursi) and the Greek-American Family H kindreds. One unaffected family member carried the mutation haplotype without the c.209A mutation, strongly suggesting its *de novo* occurrence within this family. Furthermore, a novel mutation c.488G > A (p.Arg163His; R163H) in the presenilin-2 (*PSEN2*) gene was detected, but was not associated with disease state.

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1. Introduction

Parkinson's disease (PD) is defined by the clinical signs of muscular rigidity, bradykinesia, impaired postural reflexes and, in a majority of patients, resting tremor [1,2]. Cell loss and gliosis in the substantia nigra and the presence of Lewy bodies (LB) at autopsy confirm the diagnosis [1]. In addition, the underlying neurodegenerative process may cause a variety of associated symptoms including autonomic nervous system disturbances, cognitive impairment and sleep rhythm abnormalities. PD is non-hereditary in the majority of cases, but kindreds with hereditary forms have been long reported, particularly in Sweden by Herman Lundborg in 1913 [3] and by Henry Mjönes in 1949 [4].

Golbe et al. described a large Italian-American kindred with autosomal dominant parkinsonism originating from the town of Contursi (southern Italy) [5], and Markopoulou et al. reported a similar phenotype from the Greek-American Family H [6]. In 1997, the A53T (p.Ala53 Th, c.209G > A) mutation in the α-synuclein gene (*SNCA*) was found to be associated with PD in members of the Contursi kindred and in three families from Greece [7]. The same mutation has also been identified in Family H.[8] This discovery for the first time linked a gene mutation to PD. Subsequent work revealed that the α-synuclein protein is a principal component of LB in brains from patients with α-synuclein A53T mutation [9] as well as in sporadic PD [10]. A haplotype segregating with the disease was identical in Contursi and Greek patients, suggesting a common founder [11].

The α-synuclein A53T mutation has since been detected in several additional Greek families [12–14] and in patients of Greek origin residing in Australia [15] and Germany [16]. Only three individuals without known Greek or Italian ancestry have so far been reported to carry this mutation: One patient from the United Kingdom, now deceased, displayed symptoms consistent with sporadic late-onset PD [17]. DNA from this patient was not available for haplotype analysis and contact with relatives has been lost [18]. More recently two affected members of a Korean family were studied [19], and their haplotype differed from the Greek/Contursi haplotype [19].

In vitro, α-synuclein proteins with the A53T mutation are more prone to form fibrils than wild type α-synuclein [20]. To our knowledge, no biomarker data on the evolution of the neurodegenerative process elicited by this mutation *in vivo* have so far been available.

Herein, we report a family from southern Sweden with α-synuclein A53T mutation. We present for the first time clinical, magnetic resonance imaging (MRI), single-photon emission computed tomography (SPECT), and cerebrospinal fluid (CSF)-biomarker data compiled during a 5 and 10 year longitudinal follow-up of two affected family members. We performed haplotype analysis of this family and the Greek-American Family H, whose haplotype had not yet been determined, and compared it with the previously reported Contursi–kindred haplotype.

2. Methods

2.1. Samples

Swedish PD patients (n = 99) and unaffected subjects (n = 56; spouses and siblings of probands) were enrolled in an ongoing clinical genetic research study. Of the affected probands, 61 resided in a confined geographical area (including the Lister peninsula in southern Sweden, from where we previously reported a kindred with *SNCA* duplication and triplication) [21,22]. The remainder (n = 38), were from other areas of southern Sweden and had a first- or second-degree relative with PD. The study was approved by the Institutional Review Board and written informed consent was obtained from all participants.

2.2. Genetic analyses

Genomic DNA was extracted from peripheral blood lymphocytes at Region Skåne Competence Centre, Malmö University Hospital, Sweden, using standard protocols. Leucine rich repeat kinase-2 (Lrrk2) Gly2019Ser, and Tyr1699Cys mutations as well as α-synuclein Ala30Pro, Glu46Lys and Ala53Thr (A53T) mutations and gene dosage were analyzed by TaqMan™ chemistry as described elsewhere [23]. Samples positive for the α-synuclein A53T mutation were confirmed by sequencing. PCR products were purified from unincorporated nucleotides using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Sequence analysis was conducted as previously described [23].

Haplotype analysis was performed on samples from a family with two affected members who carried an *SCNA* c.209G > A mutation (Fig. 1), the Greek-American Family H and the Contursi kindred. Genotypes were normalized to the CEPH (Centre d'Étude du Polymorphisme Humain) database (http://www.cephb.fr/en/cephdb/browser.php). Eighteen microsatellite markers spanning the *SNCA* locus and the adjacent areas on chromosome 4 were used (Fig. 1).

For the proband, PCR amplicons of the genes for microtubule-associated protein tau (*MAPT*, exon 9–13), progranulin (*PGRN*), presenilin 1 (*PSEN1*, exon 3–12), presenilin-2 (*PSEN2*, exon 3–12) and amyloid precursor protein (*APP*, exon 16–17), including intron/exon boundaries, were sequenced using ABI Big Dye Terminator v. 1.1, Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) and an ABI prism 3130xl Gene Analyzer (Applied Biosystems Inc.). Sequencing results of the *PSEN2* gene in DNA from 170 individuals with dementia and suspected heredity for dementia from southern Scandinavia (Denmark and Sweden) were used for comparison. Apolipoprotein E (*apoE*) genotyping was performed in DNA from the proband and her mother (II:5) with TaqMan™ allelic discrimination. Data were analysed with Sequencing Analysis Software Version 5.2 from Applied Biosystems, and mutation screening was performed using Mutation Surveyor v.3.1 software (SoftGenetics Mutation Surveyor). Primer sequences are available on request.

2.3. Medical and family history

Medical records of individuals II:4 and III:1 were reviewed. With the proband's and her family's informed consent, nine relatives were contacted and interviewed in person or via telephone. A Swedish translation (by A.P.) of a validated telephone questionnaire [24] was used to establish whether interviewees had signs or symptoms of PD. The pedigree was drawn with information from family members and publicly available sources.

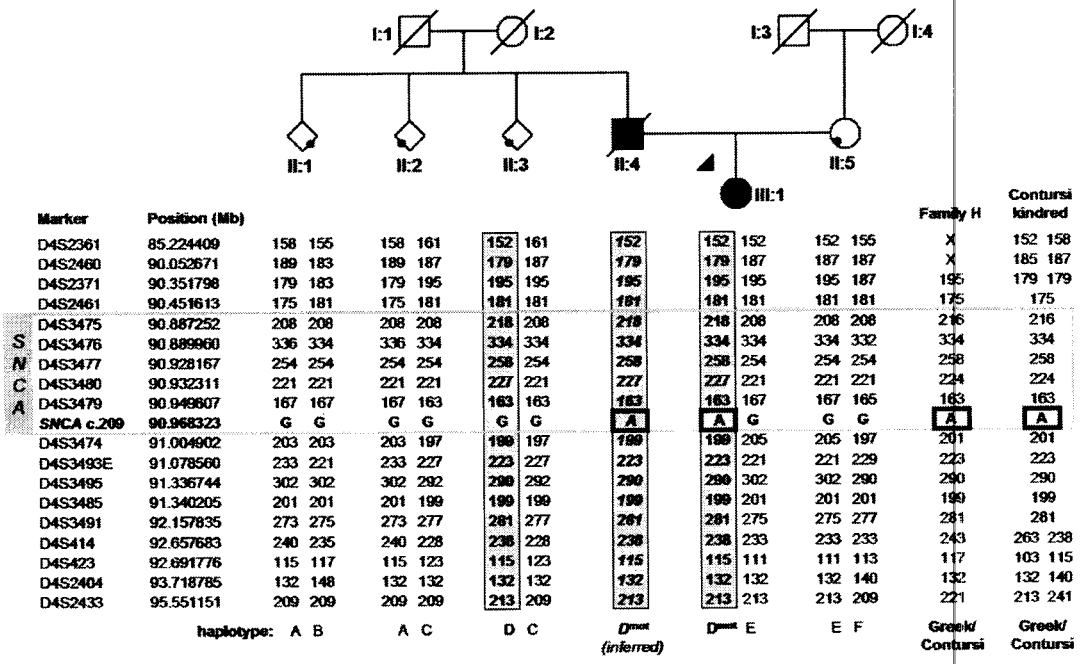
2.4. Clinical studies

Comprehensive general and neurological examinations were conducted. Brain MRI of III:1 was obtained at age 43 (years) in a 1.5T scanner and at age 45, 46 and 47 in a 3T scanner with different protocols. Transversal T2-weighted images were acquired at each time point. Other sequences included diffusion weighted imaging, transversal or coronal fluid-attenuated inversion recovery sequences, a sagittal T1 or T2-weighted sequence, and at age 47 a high resolution T1 weighted sequence. SPECT using ^{123}I-FP-CIT ([^{123}I]-2beta-carbomethoxy-3beta-(-4-iodophenyl)-N-(3-fluoropropyl)-nortropane) and regional cerebral blood flow (rCBF) assessment with SPECT using ^{99m}Tc-d,l-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO, exametazime) were performed according to standard clinical procedures. CSF was analysed for the neurochemical biomarkers indicated in Table 1 by the laboratories of the Neurochemistry Section, Sahlgrenska University Hospital, Gothenborg, Sweden.

3. Results

3.1. Genetic analyses

Though Lrrk2 Gly2019Ser and Tyr1699Cys, α-synuclein Glu46-Lys and Ala30Pro mutations and *SNCA* multiplications were not found in any of the 155 individuals screened, an α-synuclein A53T mutation was detected in the proband (III:1) of the family shown in Fig. 1. Haplotype studies indicated that the proband inherited the mutated allele on a haplotype, designated D in Fig. 1, from her affected father (II:4), from whom DNA was unavailable. We found that Family H and the Contursi kindred [11] share a haplotype, but that this haplotype is different from the Swedish D haplotype. An unaffected sibling (II:3) of the proband's father carried the D haplotype without α-synuclein A53T mutation. The proband's (III:3) *apoE* genotype was ε3/ε3 and the proband's mother's (II:5) was ε2/ε3. No pathogenic mutations in the *MAPT*, *PSEN1*, *APP*, or *PGRN* genes were found, but the proband was heterozygous for a novel PSEN2 mutation (c.488G > A, GenBank accession no. NM_000447.2). This mutation is predicted to cause an amino acid change from arginine to histidine at amino acid site 163 (p.Arg163His; R163H). The patient's mother (II:5), unaffected at age 71 years, was subsequently shown to carry the same mutation,



	Marker	Position (Mb)												Family H	Contursi kindred
	D4S2361	85.224409	158	155	158	161	152	161	152	152	152	152	155	X	152 158
	D4S2460	90.052671	189	183	189	187	179	187	179	179	187	187	187	X	185 187
	D4S2371	90.351798	179	183	179	195	195	195	195	195	195	195	187	195	179 179
	D4S2461	90.451613	175	181	175	181	181	181	181	181	181	181	181	175	175
S	D4S3475	90.887252	208	208	208	208	218	208	218	218	208	208	208	216	216
N	D4S3476	90.889960	336	334	336	334	334	334	334	334	334	334	332	334	334
C	D4S3477	90.928167	254	254	254	254	258	254	258	258	254	254	254	258	258
A	D4S3480	90.932311	221	221	221	221	227	221	227	227	221	221	221	224	224
	D4S3479	90.949607	167	167	167	163	163	163	163	163	167	167	165	163	163
	SNCA c.209	90.968323	G	G	G	G	G	G	A	A	G	G	G	A	A
	D4S3474	91.004902	203	203	203	197	199	197	199	199	205	205	197	201	201
	D4S3493E	91.078560	233	221	233	227	223	227	223	223	221	221	229	223	223
	D4S3495	91.336744	302	302	302	292	290	292	290	290	302	302	290	290	290
	D4S3485	91.340205	201	201	201	199	199	199	199	199	201	201	201	199	199
	D4S3491	92.157835	273	275	273	277	281	277	281	281	275	275	277	281	281
	D4S414	92.657683	240	235	240	228	238	228	238	238	233	233	233	243	263 238
	D4S423	92.691776	115	117	115	123	115	123	115	115	111	111	113	117	103 115
	D4S2404	93.718785	132	148	132	132	132	132	132	132	132	132	140	132	132 140
	D4S2433	95.551151	209	209	209	209	213	209	213	213	213	213	209	221	213 241
		haplotype:	A	B	A	C	D	C	D^{mut} (inferred)		D^{mut}	E	E	F	Greek/ Contursi	Greek/ Contursi

Fig. 1. Family pedigree and results from haplotype analysis: Squares indicate males, circles females, and diamonds subjects of unspecified gender to protect confidentiality. Filled symbols indicate subjects diagnosed with PD. Lower-left dots identify individuals interviewed and examined in person (conducted whenever feasible) and lower-right dots identify individuals interviewed by telephone. The black arrowhead identifies the proband. Haplotype analysis results are shown below each individual, and to the right for the Greek-American Family H and the Italian-American Contursi kindred. Letters below each haplotype specify the different or reoccurring haplotypes. Haplotype D, which carries the c.209A mutation in III:1 (designated as D^{mut}) and presumably in II:4, but not in II:3, is highlighted with light grey rectangles. Individual I:1 died at age 85 years and I:2 at 93 years of age; both had no signs of Parkinson's disease according to family information and medical records (available for I:1). Five additional relatives (not shown) from generations I and II were contacted and had no signs or symptoms of PD. X, not shared.

which was entered into www.molgen.ua.ac.be database. This mutation was not found in DNA from 170 individuals with dementia and suspected heredity for dementia from southern Scandinavia.

3.2. Clinical information

The family are of Swedish origin, unaware of any Greek, Italian, other Mediterranean, or Asian ancestry, and are unrelated to the "Lister" kindred described previously from this study's data collection [21,22,25].

Table 1
Results from cerebrospinal fluid (CSF) analysis of the **proband (III:1)**: The concentration of CSF albumin was elevated in absolute value and when compared to serum-albumin concentration. CSF cell counts were normal. There were no oligoclonal bands after isoelectric focusing and no intrathecal production of IgG. CSF-light-chain neurofilament protein (NFL) concentration was normal at age 45 but slightly elevated at age 46.5, indicating neuronal degeneration. CSF-tau protein, phospho-tau, beta-amyloid, glial fibrillary acidic protein (GFAP) and S-100-protein were within normal limits. However, CSF-beta-amyloid and CSF-GFAP were nearer to the reference range boundaries at age 46.5 when disease had progressed. In **II:4**, CSF total protein was determined instead of CSF albumin, due to different laboratory routines at the time of analysis. In all samples, CSF protein was elevated (0.51 g/l to 1.03 g/l; reference 0.15–0.45 g/l) as was the number of CSF mononuclear cells (6–17 cells/mm^3; reference <5 cells/mm^3) (data not shown in Table 1). S, serum; n.d., not determined. **Bold print and asterisks** (*) identify values outside of the laboratory's reference range.

	Unit	45 years	46,5 years	Normal range
CSF-albumin	g/l	**0.67***	**0.74***	0.07–0.33
S-albumin to CSF-albumin rate		**0.017***	**0.019***	0.0021–0.0095
CSF-tau	ng/l	150	140	<400
CSF-phospo-tau	ng/l	24	24	< 60
CSF-beta-amyloid	ng/l	620	480	>450
CSF-NFL	ng/l	<250	**400***	<250
CSF-GFAP	ng/l	420	590	<750
CSF-S-100	µg/l	*n.d.*	0,16	<1,7

3.2.1. III:1

The proband has been followed at our clinics for five years and has been examined by H.W., A.P. and C.N. At age 43, she experienced a decreased range of motion, stiffness and hypokinesia in her right arm and heaviness in her right leg. She indicated that the symptoms started insidiously when she was 39–41 years old. About six months after the onset of motor symptoms, she noted difficulties finding simple words, and sometimes would not finish a sentence. Diction was monotonous, with occasional stuttering. Pramipexole improved the rigidity. At age 43, she was unable to work and became increasingly unable to perform household chores. At age 44, she also had developed difficulty initiating speech, and neurological evaluation revealed signs of motor and sensory dysphasia and dysarthria. Body bradykinesia and hypomimia had become pronounced, and rigidity as well as a slight tremor was noted. At age 45 years, there remained a positive, albeit limited, effect of dopaminergic treatment (500 mg/d levodopa, 1000 mg/d entacapone, and 0.54 mg/d pramipexole), but dyskinesias were observed. Urological examination revealed a neurogenic bladder disturbance, which was alleviated with desmopressin 60 µg/d. At the most recent examination at age 47, the patient's speech was largely unintelligible due to hypophonia and rapid rate. In motor "ON" state, the patient's gait was peculiar as she set her feet directly in front of each other (as in tandem gait) or even slightly to the opposite side of the midline, "rolling" her trunk from side to side. During "OFF"-state, she had difficulties with gait initiation and shuffling gait. Spontaneous, asymmetric myoclonus was noted in the upper extremities and negative myoclonus in the hands and fingers. Eye movements were normal and there was no sensory deficit. Neuropsychological

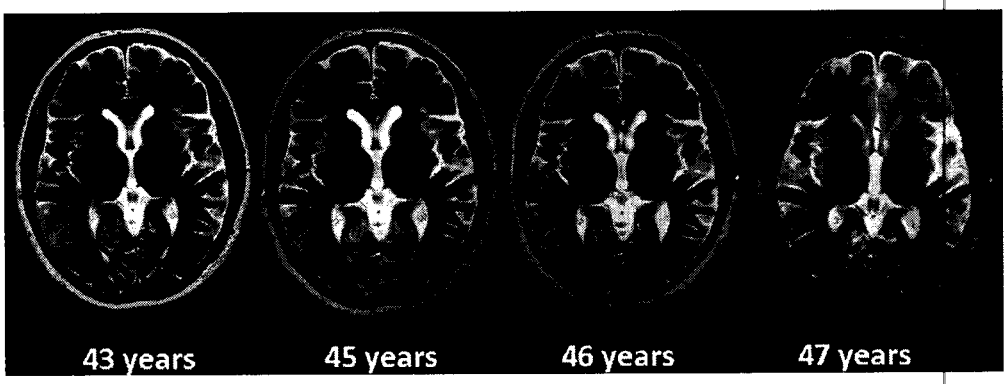


Fig. 2. MRI of the proband (III:1): T1-weighted sequence at age 43 years, fluid-attenuated inversion recovery (FLAIR) sequence at age 45, 46 and 47 years. No signal changes are present. Specifically, the basal ganglia are normal and there is no definite focal or general atrophy.

assessment revealed decreased performance in tasks regarding abstraction, visuospatial construction and executive functioning. The Mini-Mental Status Examination score was 19/30 and dementia was diagnosed. Treatment with rivastigmine led to modest cognitive improvement. Repeat brain MRI scans were normal (Fig. 2). EEG background rhythm was 7–8 Hz, no epileptiform activity was detected. Lumbar puncture was performed twice. See Table 1 for results of CSF analyses and Fig. 3 for SPECT examinations.

3.2.2. II:4

The proband's father developed resting tremor in his right hand before age 30 years. At age 32 years, he developed imbalance and falls secondary to symptomatic orthostatic hypotension, dysdiadochokinesia, slight generalized rigidity, diminution of facial expression, and myoclonic jerks in the right thumb. He began to walk stiffly and uneasily, and the tremor in his right hand had become more continuous, rendering manual work impossible. EEG background rhythm was 7–8 Hz, epileptic acvtivity was absent. Speech difficulties had arisen at age 33, described as "words stick in the mouth", with the patient "stumbling over words when nervous". Electrocoagulation of the left ventrolateral thalamic nucleus during the 1960s alleviated the tremor in the right hand. However, at age 36, a left hand tremor had developed and generalized rigidity had become pronounced. At age 38, he moved to a nursing home. His speech was monotonous and difficult to understand. Bradykinesia, diplopia and dysconjugate gaze as well as urinary incontinence were noted. Levodopa treatment had a positive effect on bradykinesia and facial expression but caused dyskinesias. At age 40 years, he was aphonic, unable to follow commands, and a diagnosis of dementia was made. While standing, he had camptocormia and held his arms in flexed posture. Repeated lumbar punctures were performed over a 4-year period (caption, Table 1). During the last year of his life, he required the assistance of two aides for walking and was unable to feed himself without help. He died at age 42. An autopsy was not performed.

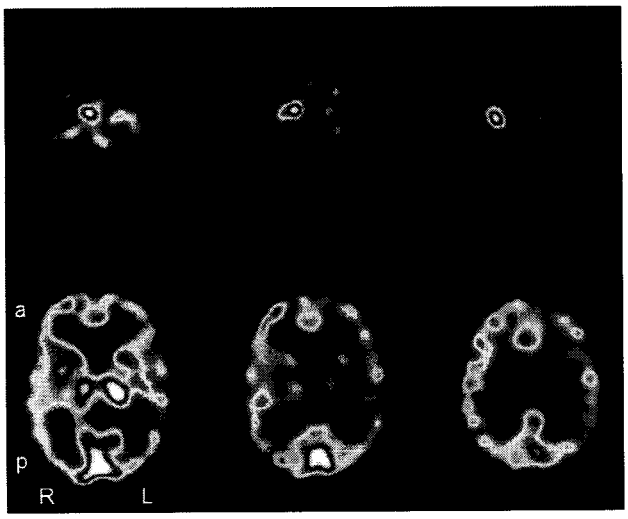


Fig. 3. SPECT of the proband (III:1): Top: ^{123}I-FP-CIT SPECT at age 45 years revealed markedly decreased presynaptic dopamine reuptake capacity in basal ganglia bilaterally, but clearly more so on left side. Bottom: ^{99m}Tc-HMPAO SPECT at age 47 years revealed reduced cortical blood flow, most markedly in the parietal lobes. This reduction was more prominent in the left hemisphere, where some reduction of cortical blood flow also occurred in the temporal lobe and the lateral portion of the frontal lobe. R, right; L, left; a, anterior; p, posterior.

4. Discussion

Herein, we report a family from southern Sweden with an α-synuclein A53T mutation. The clinical characteristics in the two affected individuals included an early disease manifestation before age 41 and 30 years, rapid progression to a severe phenotype with tremor, rigidity, bradykinesia and gait disturbance, and an initially good response to levodopa treatment. Language and speech difficulties occurred relatively early in the course of the disease, and were followed by cognitive decline. Myoclonic jerks were documented in both individuals.

The proband (III:1) and an unaffected relative (II:3) share identical haplotypes except for the presence of the c.209A mutation in the proband. Although theoretically possible, we consider it highly improbable that I:1 or I:2 would have carried the A53T mutation but remained asymptomatic until their death at age 85 and 93 years. It is impossible to confirm whether II:4 carried the mutation as DNA was not available; however, the parkinsonian symptoms of both II:4 and III:1 were highly similar and have not been reported in any other family member. We conclude that the mutation occurred *de novo* between generation I and II. We consider these findings the strongest evidence so far that this mutation is sufficient by itself to cause disease.

Cognitive impairment like that seen in the affected members of this family has been reported previously in α-synuclein A53T-associated PD [5,6,14,15,26,27]. However, the severity of cognitive dysfunction was highly variable, occurring early [28] or late [5,28] during the disease course, and several A53T patients remained

cognitively intact [12,29]. Language and speech impairment has also been found in other A53T patients [6,12,19,26,28,29]. Previous reports also identified prominent myoclonus [6, 15], severe orthostatic hypotension [15,26–28], and neurogenic bladder disturbance [15]. These also occurred in this family, and interestingly, in disease associated with SNCA multiplication [21]. The age at symptom onset was highly variable in published reports, spanning the interval from 20 to 85 years [7], with means of 45.6 [7] and 47.9 years [27]. Thus, the two patients reported here have an early onset of symptoms.

Since both the proband and her father developed dementia, we analyzed genes implicated in hereditary dementia. We found a novel mutation c.488G > A (p.Arg163His; R163H) in the presenilin-2 (*PSEN2*) gene. This mutation was absent in 170 individuals from the same geographical area (southern Scandinavia) who had been examined genetically for suspected hereditary dementia. Thus, the mutation is rare and not commonly associated with hereditary dementia in this population. A modifying effect of the presenilin-2 R163H mutation in individuals with α-synuclein A53T mutation cannot be excluded with certainty. However, the presenilin-2 R163H mutation was also present in the proband's unaffected mother. We thus suggest that this mutation is a non-pathogenic variant without clinical significance. As DNA was only available from one affected person, no other genetic factors were analysed. A study of members of different families with the α-synuclein A53T mutation could help elucidate whether other genetic factors contribute to phenotypic variability in A53T-related PD.

Our present understanding of the pathogenic effects of the α-synuclein A53T mutation has come from clinical descriptions, genetic analyses, and neuropathological examinations. Here, we present longitudinal clinical and biomarker data from individuals II:4 and III:1, obtained over the course of 10 and 5 years, respectively. II:4 was examined repeatedly at our institution in the 1960s and 70s, III:1 during the years prior to this publication. In both patients, the background rhythm was reduced in EEGs performed 2 (II:4) and 4 years (III:3) after the onset of symptoms. A previous report of EEG results from one patient with A53T mutation showed bitemporal slowing with hyperventilation but a normal background rhythm [6]. Repeated brain MRI studies were normal in the proband. Previous reports indicate that cranial CT [6,13] or MRI [13,14] are normal in A53T patients, and one report of mild cerebral atrophy was ascribed to old age [17]. Both III:1 and II:4 exhibited elevated CSF–protein or albumin concentrations, with repeated measurements showing two to four times the mean reference value. CSF mononuclear cells were elevated in all CSF samples analyzed from II:4, but not III:1. In III:1's second lumbar puncture (performed 17 months after the first), the concentration of CSF–light-chain neurofilament protein (NFL), a structural axonal protein, was elevated, while the concentration of beta-amyloid(1–42) was considerably lower, possibly reflecting the evolution of the underlying pathological process. CSF-NFL is considered to aid in differentiating PD, where it is normal, from multiple system atrophy (MSA), where it is elevated. Our results suggest that NFL elevation may simply reflect the extent and rate of neurodegeneration. An ^{123}I-FP-CIT SPECT analysis in III:1 identified clearly reduced dopamine reuptake capacity and cortical blood flow (Fig. 3). Blood flow reduction was most marked in the dominant hemisphere, consistent with the observed language deficits.

These results suggest that there is an underlying diffuse encephalopathic and/or neurodegenerative process in α-synuclein A53T-associated disease which affects the cerebral cortex and dopaminergic system, with increased vascular wall permeability causing protein leakage into the CSF, cell death, decreased cortical blood flow, dopamine depletion and slowed EEG background rhythm. These findings are consistent with the abundant cortical α-synuclein deposition found in *post mortem* examinations of the brains of α-synuclein A53T-positive individuals [15,28,30].

This study is limited by the low number (two) of affected individuals. There may be alternative explanations for the increase in CSF cell count and protein or albumin levels, such as a gliotic reaction to the neurosurgical treatment in II:4, a low-level asymptomatic infectious disease, or another unknown cause. Repeated lumbar puncture by itself is known to cause slight elevation of CSF-protein and cell count, although this does not explain why both values were raised in the very first examination in both individuals. Additional clinical data from other A53T individuals will reveal whether these conclusions can be applied generally.

5. Conclusion

The α-synuclein A53T mutation leads to a characteristic parkinsonian syndrome with varying degree of cognitive dysfunction. This point mutation, as well as genomic *SNCA* multiplications, may cause disease by increased α-synuclein aggregation in different brain regions. Patients with these mutations may be ideal candidates for clinical trials with inhibitors of α-synuclein expression and aggregation when such agents become available. Prospective biomarker studies on individuals with these mutations would be valuable to elucidate whether there is a common, α-synuclein mediated pathway in the pathogenesis of all forms of idiopathic PD (for which disease caused by different *SNCA* mutations would be a highly suitable model) or if a variety of different pathological processes are associated with clinical phenotypes that meet the diagnostic criteria for PD.

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Conflict of interest

Authors report no conflict of interests.

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