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

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Swedish de novo α-synuclein A53T mutation

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Abstract:
A *de novo* α-synuclein A53T (p.Ala53Thr; 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.

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.Ala53Thr, 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 (Figure 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 (Figure 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 (Sweden and Denmark) 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]-2 beta-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 Glu46Lys 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 Figure 1. Haplotype studies indicated that the proband inherited the mutated allele on a haplotype, designated D in Figure 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, which was entered into the 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].

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 (500mg/d levodopa, 1000mg/d entacapone, and 0.54mg/d pramipexole), but dyskinesias were observed. Urological examination revealed a neurogenic bladder disturbance, which was alleviated with desmopressin 60µg/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 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 (Figure 2). EEG background rhythm was 7-8Hz, no epileptiform activity was detected. Lumbar puncture was performed twice. See Table 1 for results of CSF analyses and Figure 3 for SPECT examinations.

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 7-8Hz, 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 1960's 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.

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 (Figure 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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References

[1] Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. Archives of neurology. 1999 Jan;56(1):33-9.

[2] Calne DB, Snow BJ, Lee C. Criteria for diagnosing Parkinson's disease. Annals of neurology. 1992;32 Suppl:S125-7.

[3] Lundborg H. Medizinisch-biologische Familienforschungen innerhalb eines 2232köpfigen Bauerngeschlechtes in Schweden (Provinz Blekinge). Jena: Verlag von Gustav Fischer 1913.

[4] Mjönes H. Paralysis agitans. A clinical and genetic study. Acta Psychiatrica et Neurologica Supplementum. 1949;54:195.

[5] Golbe LI, Di Iorio G, Bonavita V, Miller DC, Duvoisin RC. A large kindred with autosomal dominant Parkinson's disease. Annals of neurology. 1990 Mar;27(3):276-82.

[6] Markopoulou K, Wszolek ZK, Pfeiffer RF. A Greek-American kindred with autosomal dominant, levodopa-responsive parkinsonism and anticipation. Annals of neurology. 1995 Sep;38(3):373-8.

[7] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science (New York, NY. 1997 Jun 27;276(5321):2045-7.

[8] Markopoulou K, Wszolek ZK, Pfeiffer RF, Chase BA. Reduced expression of the G209A alpha-synuclein allele in familial Parkinsonism. Annals of neurology. 1999 Sep;46(3):374-81.

[9] Langston JW, Sastry S, Chan P, Forno LS, Bolin LM, Di Monte DA. Novel alpha-synuclein-immunoreactive proteins in brain samples from the Contursi kindred, Parkinson's, and Alzheimer's disease. Experimental neurology. 1998 Dec;154(2):684-90.

[10] Mezey E, Dehejia AM, Harta G, Tresser N, Suchy SF, Nussbaum RL, et al. Alpha synuclein is present in Lewy bodies in sporadic Parkinson's disease. Molecular psychiatry. 1998 Nov;3(6):493-9.

[11] Athanassiadou A, Voutsinas G, Psiouri L, Leroy E, Polymeropoulos MH, Ilias A, et al. Genetic analysis of families with Parkinson disease that carry the Ala53Thr mutation in the gene encoding alpha-synuclein. American journal of human genetics. 1999 Aug;65(2):555-8.

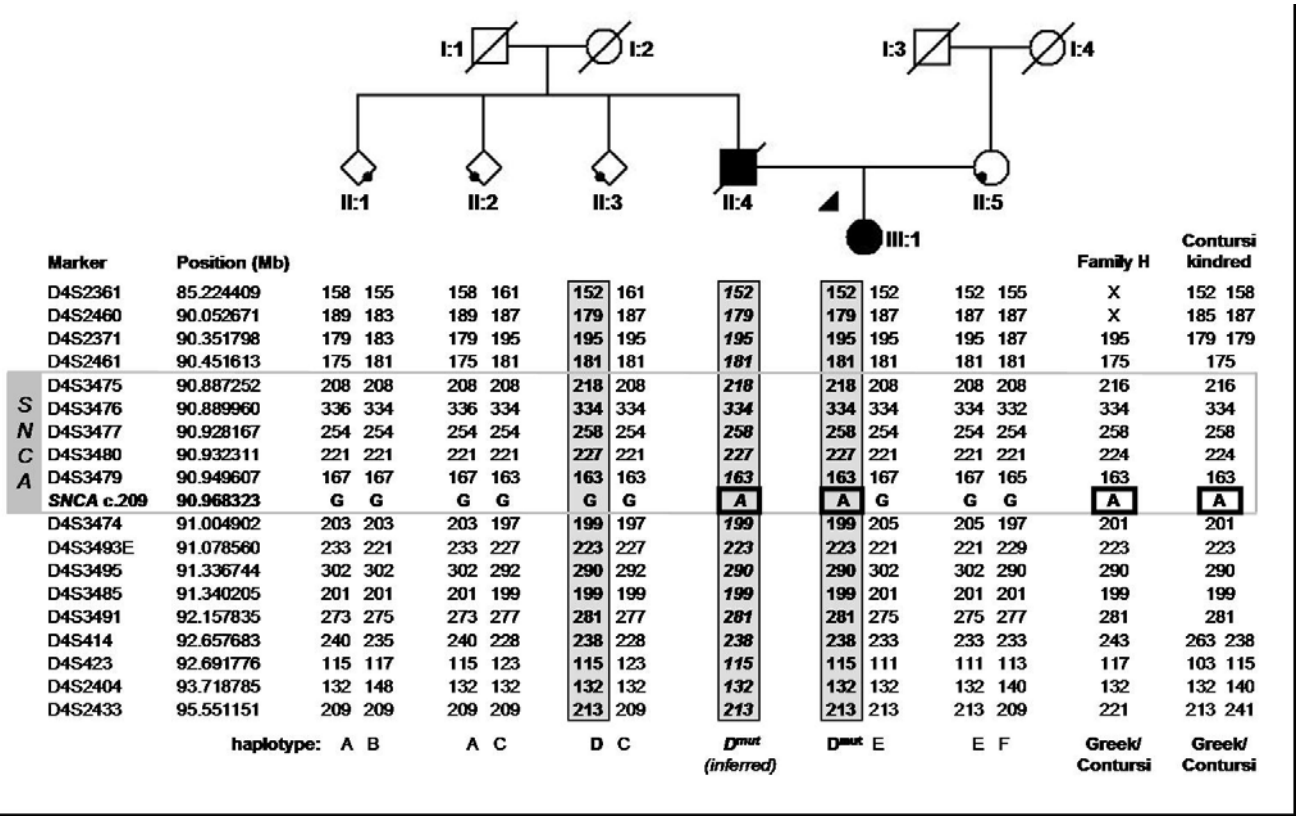
[12] Veletza S, Bostatzopoulou S, Hantzigeorgiou G, Kazis A, Papadimitriou A. α-Synuclein mutation associated with familial Parkinson’s disease in two new Greek kindreds (abstract). J Neurol. 1999;246(suppl1):1-43.

[13] Papadimitriou A, Veletza V, Hadjigeorgiou GM, Patrikiou A, Hirano M, Anastasopoulos I. Mutated alpha-synuclein gene in two Greek kindreds with familial PD: incomplete penetrance? Neurology. 1999 Feb;52(3):651-4.

[14] Bostantjopoulou S, Katsarou Z, Papadimitriou A, Veletza V, Hatzigeorgiou G, Lees A. Clinical features of parkinsonian patients with the alpha-synuclein (G209A) mutation. Mov Disord. 2001 Nov;16(6):1007-13.

[15] Spira PJ, Sharpe DM, Halliday G, Cavanagh J, Nicholson GA. Clinical and pathological features of a Parkinsonian syndrome in a family with an Ala53Thr alpha-synuclein mutation. Annals of neurology. 2001 Mar;49(3):313-9.

[16] Berg D, Niwar M, Maass S, Zimprich A, Moller JC, Wuellner U, et al. Alpha-synuclein and Parkinson's disease: implications from the screening of more than 1,900 patients. Mov Disord. 2005 Sep;20(9):1191-4.
[17] Michell AW, Barker RA, Raha SK, Raha-Chowdhury R. A case of late onset sporadic Parkinson's disease with an A53T mutation in alpha-synuclein. Journal of neurology, neurosurgery, and psychiatry. 2005 Apr;76(4):596-7.
[18] Michell AW. Personal communication 2008 October 22. Cited with permission.
[19] Ki CS, Stavrou EF, Davanos N, Lee WY, Chung EJ, Kim JY, et al. The Ala53Thr mutation in the alpha-synuclein gene in a Korean family with Parkinson disease. Clinical genetics. 2007 May;71(5):471-3.
[20] Conway KA, Harper JD, Lansbury PT. Accelerated in vitro fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. Nature medicine. 1998 Nov;4(11):1318-20.
[21] Fuchs J, Nilsson C, Kachergus J, Munz M, Larsson EM, Schule B, et al. Phenotypic variation in a large Swedish pedigree due to SNCA duplication and triplication. Neurology. 2007 Mar 20;68(12):916-22.
[22] Puschmann A, Wszolek ZK, Farrer M, Gustafson L, Widner H, Nilsson C. Alpha-synuclein multiplications with parkinsonism, dementia or progressive myoclonus? Parkinsonism Relat Disord. 2009 June;15(5):390-2.
[23] Mata IF, Ross OA, Kachergus J, Huerta C, Ribacoba R, Moris G, et al. LRRK2 mutations are a common cause of Parkinson's disease in Spain. Eur J Neurol. 2006 Apr;13(4):391-4.
[24] Rocca WA, Maraganore DM, McDonnell SK, Schaid DJ. Validation of a telephone questionnaire for Parkinson's disease. Journal of clinical epidemiology. 1998 Jun;51(6):517-23.
[25] Puschmann A. Unverricht-Lundborg disease-A misnomer? Mov Disord. 2009 March 15;24(4):629-30.
[26] Golbe LI, Di Iorio G, Sanges G, Lazzarini AM, La Sala S, Bonavita V, et al. Clinical genetic analysis of Parkinson's disease in the Contursi kindred. Annals of neurology. 1996 Nov;40(5):767-75.
[27] Papapetropoulos S, Ellul J, Paschalis C, Athanassiadou A, Papadimitriou A, Papapetropoulos T. Clinical characteristics of the alpha-synuclein mutation (G209A)-associated Parkinson's disease in comparison with other forms of familial Parkinson's disease in Greece. Eur J Neurol. 2003 May;10(3):281-6.
[28] Markopoulou K, Dickson DW, McComb RD, Wszolek ZK, Katechalidou L, Avery L, et al. Clinical, neuropathological and genotypic variability in SNCA A53T familial Parkinson's disease. Variability in familial Parkinson's disease. Acta neuropathologica. 2008 Jul;116(1):25-35.
[29] Kotzbauer PT, Giasson BI, Kravitz AV, Golbe LI, Mark MH, Trojanowski JQ, et al. Fibrillization of alpha-synuclein and tau in familial Parkinson's disease caused by the A53T alpha-synuclein mutation. Experimental neurology. 2004 Jun;187(2):279-88.
[30] Duda JE, Giasson BI, Mabon ME, Miller DC, Golbe LI, Lee VM, et al. Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. Acta neuropathologica. 2002 Jul;104(1):7-11.



	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
SNCA	D4S3475	90.887252	208	208	208	208	218	208	*218*	218	208	208	208	216	216
SNCA	D4S3476	90.889960	336	334	336	334	334	334	*334*	334	334	334	332	334	334
SNCA	D4S3477	90.928167	254	254	254	254	258	254	*258*	258	254	254	254	258	258
SNCA	D4S3480	90.932311	221	221	221	221	227	221	*227*	227	221	221	221	224	224
SNCA	D4S3479	90.949607	167	167	167	163	163	163	*163*	163	167	167	165	163	163
SNCA	***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 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 in **bold print**. 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.

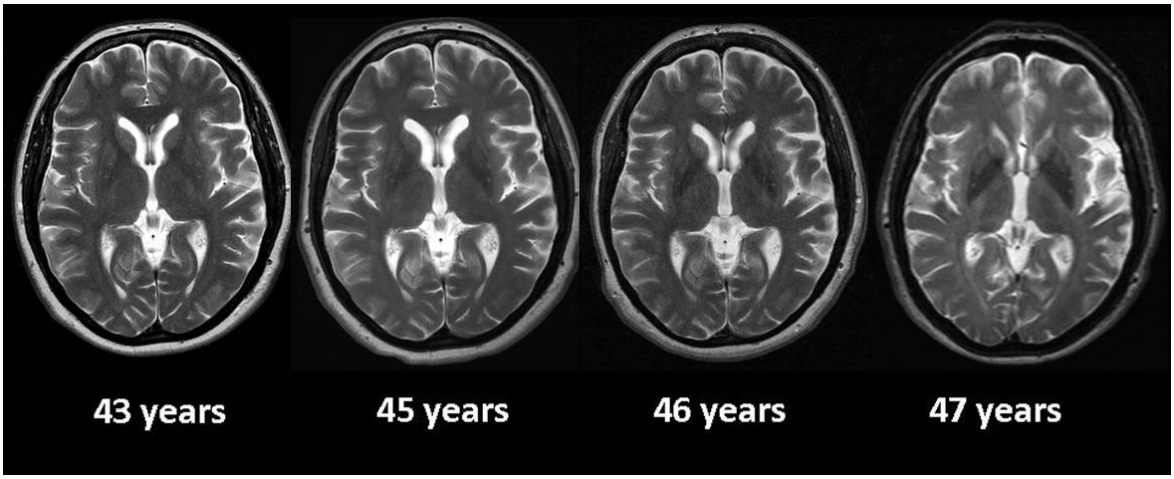


Fig. 2. MRI of the proband (III:1): T1-weighted sequence at age 43 years, fluid-attenuated inversion recovery (FLAIR) sequence at age 45, 46 and 47 years. No signal changes are present. Specifically, the basal ganglia are normal and there is no definite focal or general atrophy.

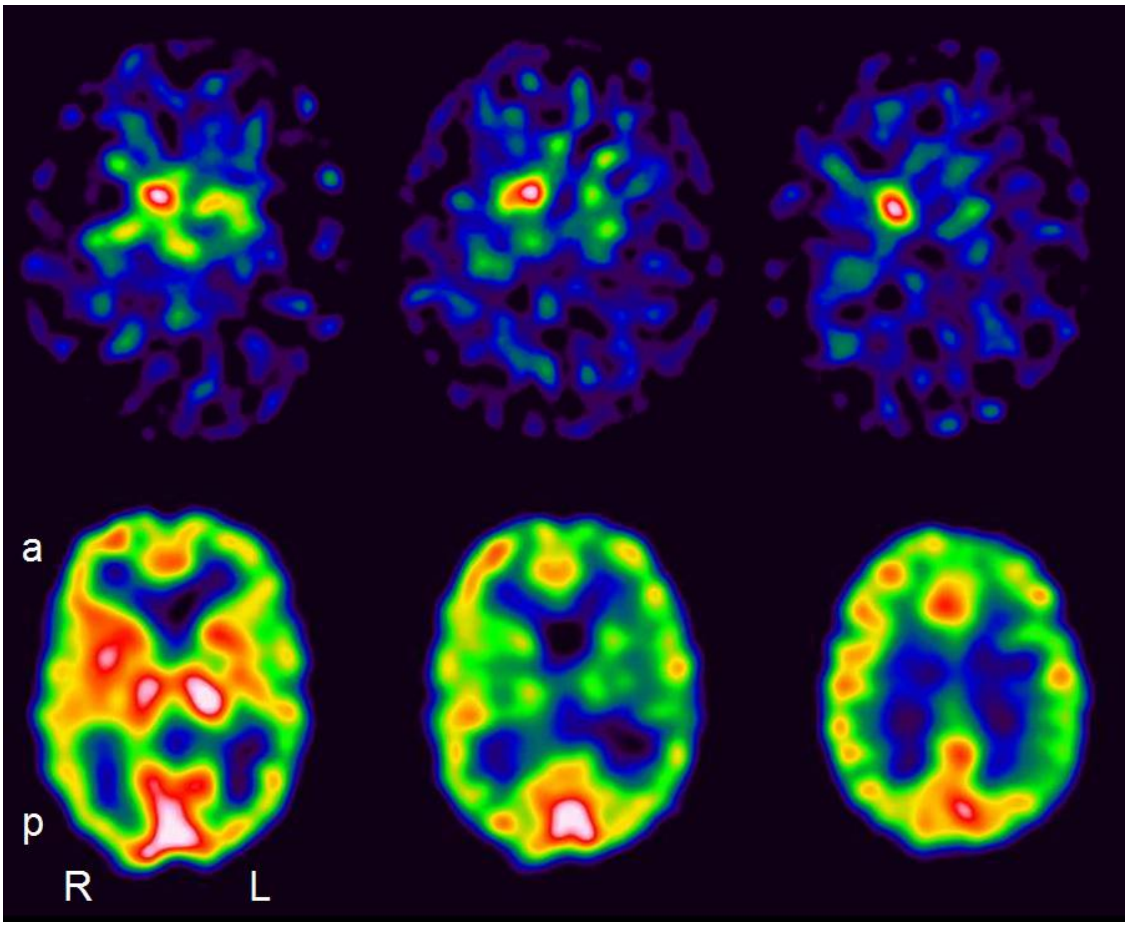


Fig. 3. SPECT of the proband (III:1): Top: ^{123}I-FP-CIT SPECT at age 45 years revealed markedly decreased presynaptic dopamine reuptake capacity in basal ganglia bilaterally, but clearly more so on left side. Bottom: ^{99m}Tc-HMPAO SPECT at age 47 years revealed reduced cortical blood flow, most markedly 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.

	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

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 (0.51g/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.