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Reduced Striatal [^{123}I]FP-CIT Binding in SCA2 Patients without Parkinsonism

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Degeneration of substantia nigra has been described in spinocerebellar ataxia type 2 (SCA2). In this study, dopamine transporter (DAT) density with [^{123}I]FP-CIT SPECT was studied in six SCA2 patients with no parkinsonian signs, six Parkinson's disease (PD) patients, and six controls. Marked striatal DAT loss was found in both SCA2 and PD patients. However, a more severe reduction in the caudate and a higher putamen to caudate ratio distinguished SCA2 from PD patients, suggesting a more uniform nigrostriatal impairment in SCA2. Striatal DAT density of SCA2 patients correlated with the severity of cerebellar ataxia.

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Autosomal dominant cerebellar ataxias (ADCAs) are progressive neurodegenerative disorders characterized by degeneration of cerebellum, brainstem, and spinal cord.[1] Twenty loci have been identified (www.geneclinics.org). Spinocerebellar ataxia type 2 (SCA2) is the second most common form of ADCAs, accounting for approximately 15% of cases in Europe, United States, and China.[2–4] SCA2 is characterized by progressive gait and limb ataxia, dysarthria, slow saccades, supranuclear ophthalmoplegia, decreased or absent tendon reflexes, and dementia. The mutation responsible for the disease is an unstable CAG trinucleotide repeat expansion within the coding sequence of the *SCA2* gene (MIM 183090), encoding for a novel protein of approximately 140kDa ataxin-2 (MIM 601517) that has a cytoplasmic localiza-

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tion in the Golgi apparatus. Typical neuropathological changes occur in the cerebellar cortex, pontine nuclei, and inferior olivary nucleus (olivopontocerebellar atrophy), but marked degeneration is also present in the substantia nigra and the spinal cord.[5–7] Despite marked involvement of the extrapyramidal system, SCA2 is typically not associated with parkinsonism.[5,8] Tremor, usually in the form of action tremor, is frequently present[5] and associated with the CAG repeat size.[9] Bradykinesia and rigidity are absent. However, in recent studies the *SCA2* mutation was reported in patients with familial L-dopa–responsive parkinsonism, suggesting that this mutation may be responsible for a subset of familial parkinsonism.[10–14] These recent studies, along with previous neuropathological studies, have highlighted the existence of dopaminergic dysfunction associated with SCA2.

This study was aimed at evaluating the presence and severity of nigrostriatal dopaminergic dysfunction in SCA2 patients without parkinsonism. The objectives were to quantify *in vivo*, with [^{123}I]FP-CIT single-photon emission computed tomography (SPECT), the degree of dopaminergic terminal loss in SCA2 and to compare it with that of idiopathic Parkinson's disease (PD).

Subjects and Methods

Subjects

Six SCA2 patients, six PD patients, and six controls were studied. Clinical and genetic data of SCA2 patients are reported in the Table. SCA2 patients presented with mild-to-moderate disease. They were clinically evaluated with the International Cooperative Ataxia Rating Scale (ICARS)[15] and the Inherited Ataxia Progression Scale[16] that classifies patients in four stages (I: asymptomatic; II: symptoms present but mild; III: symptoms completely developed; IV wheelchair-bound). Genetic analysis was performed as reported previously.[9] All patients were ambulant and in none of them were parkinsonian features (bradykinesia, rigidity and rest tremor) or cognitive decline assessed on standard neurological evaluation evident.

Table. Clinical Data, Genetics, and Striatal [^{123}I]FP-CIT Specific to Nondisplaceable Ratio (V_3'') of Patients with Spinocerebellar Ataxia Type 2 (SCA2) and Parkinson's Disease (PD)

Patient No./Sex	Age (yr)	Disease Duration (yr)[b]	CAG Repeats	More Affected Side,[c] Extrapyramidal Signs, Pyramidal Signs, Bulbar Signs, Peripheral Neuropathy[d]	IAPS[e]	ICARS Total (subscores I, II, III, IV)[f]	Striatal V_3''
SCA2 patients							
1/M	44	5	39/22	Right, n, n, y, n	2	22 (10, 8, 3, 1)	1.13
2/M	45	8	38/22	Both, n, n, y, y	2	14 (8, 3, 2, 1)	1.37
3/M	47	13	39/22	Both, n, n, n, y	2	32 (14, 12, 4, 2)	1.05
4[a]/M	56	16	38/22	Left, n, n, y, n	2	22 (8, 10, 3, 1)	0.97
5/M	45	10	38/22	Right, n, n, n, y	2	29 (13, 12, 3, 1)	1.14
6[a]/F	54	12	38/27	Right, n, y, y, y	3	40 (22, 13, 4, 1)	0.74
Mean ± SD	48 ± 5	10 ± 4	—	—	2.2 ± 0.4	27 ± 9[g]	1.07 ± 0.21[h]
PD patients					Hoehn and Yahr Stage	UPDRS-motor	
1/M	43	2		Right, y, n, n, n	1.5	10	1.31
2/F	47	5		Right, y, n, n, n	2	16	1.11
3/M	41	4		Right, y, n, n, n	2	22	1.62
4/F	43	9		Left, y, n, n, n	3	35	0.87
5/F	47	9		Left, y, n, n, n	3	18	1.38
6/F	42	4		Left, y, n, n, n	2	26	1.28
Mean ± SD	44 ± 2	6 ± 3		—	2.3 ± 0.6	21.2 ± 8.7	1.26 ± 0.25[h]
						Controls Mean ± SD	2.75 ± 0.20

[a]Patients 4 and 6 were siblings.
[b]Disease duration was calculated as the interval between onset of symptoms and day of SPECT scan.
[c]Laterality of the disease was evaluated on subscore II of the ICARS (see below) in SCA2 patients and on Unified Parkinson's Disease Rating Scale part III (UPDRS-motor) and clinical examination in PD patients.
[d]Presence of any of the listed signs are reported (y = yes, n = no).
[e]Stage of the Inherited Ataxia Progression Scale (IAPS) is reported.
[f]Total score of the International Cooperative Ataxia Rating Scale (ICARS) is reported. Subscores for posture and gait disturbances (I), kinetic functions (II), speech disorders (III), and oculomotor disorders (IV) are listed in parentheses.
[g]Mean ± SD are shown only for total ICARS score.
[h]Significantly lower than controls, $p < 0.001$ by one-way analysis of variance.

PD = Parkinson's disease; SD = standard deviation.

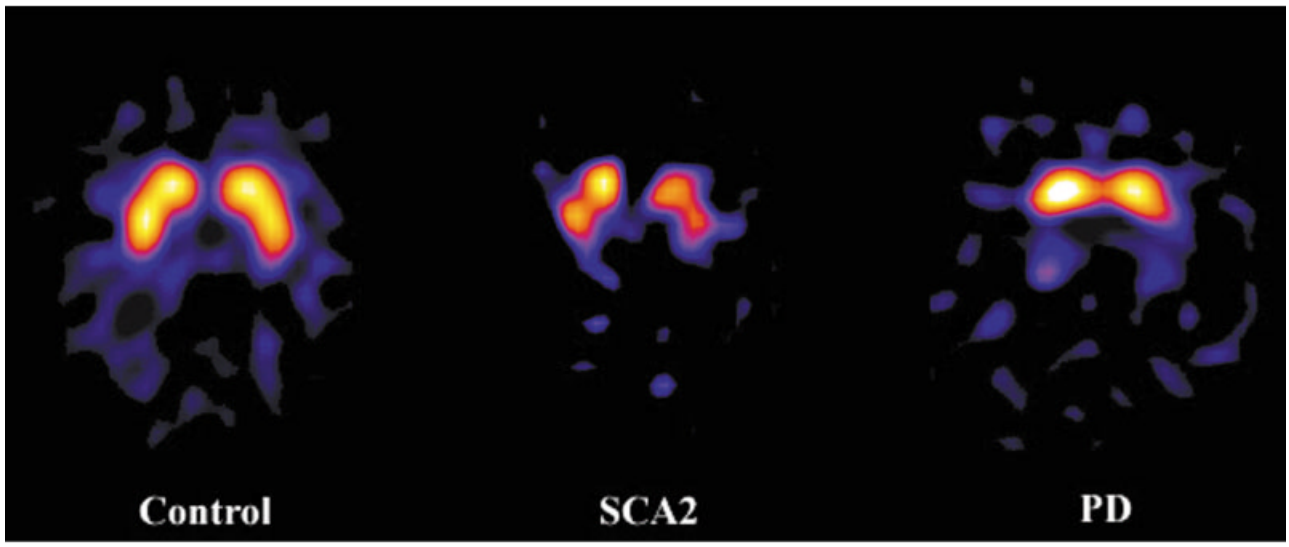

Fig 1. Transaxial [^{123}I]FP-CIT single-photon emission computed tomography scans at the level of basal ganglia in a control (left), a SCA2 patient (middle), and a Parkinson's disease patient (right). [^{123}I]FP-CIT binding was markedly reduced in both patients and showed a more uniform reduction in SCA2.

Some patients irregularly took vitamin E. No one was in therapy with amantadine or other antiparkinsonian drugs. SCA2 patients were compared with a group of PD patients with mild-to-moderate disease (clinical data are reported in the Table) who did not have any of the known *parkin* gene mutations. Healthy controls (5 men, 1 woman, age 51 ± 13 years) were recruited from spouses of affected patients and volunteers without any neurological and psychiatric disorders. All subjects gave their informed consent for the participation to the study that was approved by the local institutional review board.

Single-photon Emission Computed Tomography Studies

SPECT studies were performed 4.2 ± 0.4 hours after [^{123}I]FP-CIT intravenous injection (185 MBq) using a dual-headed camera (E.CAM; Siemens Medical Systems, Hoffmann Estates, IL) equipped with low-energy high-resolution collimators. Scans were acquired with a photopeak window centered around 159 keV ± 10% with a 128 × 128 matrix (zoom: 1.23; pixel size: 3.90 × 3.90 mm).

Images were reconstructed using a Butterworth filter (cutoff, 0.5 cycles/pixel, order, 10), corrected for attenuation using Chang's algorithm ($\mu = 0.06\ cm^{-1}$) and then spatially normalized in the Montreal Neurological Institute space to a voxel size of 4 × 4 × 4 mm, using a [^{123}I]FP-CIT template generated with the scans of the first five controls and an affine transformation without nonlinear components (SPM'99; Wellcome Department of Cognitive Neurology, London, UK). Region of interest (ROI) analysis was performed on the normalized images (ImageJ; rsb.info.nih.gov/ij/, National Institutes of Health, MD). A template of four circular ROIs (32 mm^2) for right and left caudate and putamen and one polygonal ROI (3,504 mm^2) for the occipital cortex was applied on six consecutive transaxial slices (range of Z level in Montreal Neurological Institute space: −12–8 for striatum and 0–20 for occipital cortex). The outcome measure was the specific to nondisplaceable binding ratio, V_3'' ($ROI_{striatum} - ROI_{occipital}/ROI_{occipital}$), which is an indirect measure of dopamine transporter (DAT) density. Calculated values were the average striatum, caudate and putamen V_3'', the putamen to caudate ratio, the average V_3'' in the ipsilateral and contralateral striatum (relative to the more affected body side), and the striatal asymmetry index [(ipsilateral − contralateral)/mean (ipsilateral and contralateral)*100]. In the SCA2 patients with bilateral disease, right and left striata were arbitrarily assigned as ipsilateral and contralateral. Differences among groups were assessed with one-way analysis of variance, with Tukey's protected t test for post hoc analysis with multiple comparisons. Differences between striatal sides were assessed with two-tailed paired t test. Difference of striatal asymmetry index was assessed with two-tailed unpaired t test. Correlations between striatal V_3'' and clinical variables were assessed with the Pearson correlation coefficient, r. Significance of the tests was set at the p value less than 0.05 level.

Results

There were no differences of age among SCA2, PD patients, and controls ($F = 1.23$, $p = 0.32$). Striatal [^{123}I]FP-CIT uptake was markedly reduced in SCA2 and PD patients (Fig 1). DAT density in the whole striatum was reduced by 61% in SCA2 and by 54% in PD patients compared with controls ($F = 102.29$, $p < 0.0001$, see Table). In both groups caudate and putamen DAT density was reduced ($F = 72.40$ and $F = 115.02$, $p < 0.0001$). However, caudate V_3'' was decreased more in SCA2 than PD patients (60% vs 42% reduction, $p < 0.01$, Fig 2A), whereas reduction of putamen V_3'' was similar in SCA2 and PD (65% vs 67% reduction, see Fig 2A). This resulted in a putamen to caudate V_3'' ratio higher in SCA2 patients than in PD ($p < 0.01$, see Fig 2B), with no overlap of individual values between the two groups. V_3'' was significantly

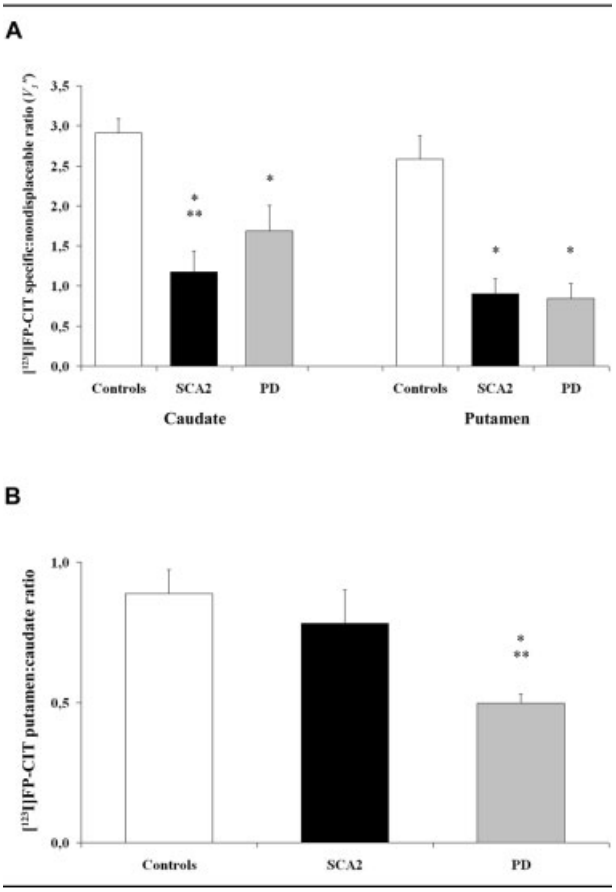

*Fig 2. Bar graphs showing [^{123}I]FP-CIT specific to nondisplaceable ratio (V_3'', A) and putamen to caudate ratio (B) in controls, SCA2, and Parkinson's disease (PD) patients. Mean ± SD are shown. In A, *p < 0.001 = significantly reduced compared with controls by one-way ANOVA; **p < 0.01 = significantly reduced compared with PD patients by Tukey's protected* t *test. In B, *p < 0.001 = significantly reduced compared with controls by one-way ANOVA; **p < 0.01 = significantly reduced compared with SCA2 patients by Tukey's protected* t *test.*

lower in the contralateral than in the ipsilateral striatum both in SCA2 (0.96 ± 0.24 vs 1.13 ± 0.17, $p < 0.01$) and PD patients (1.10 ± 0.27 vs 1.42 ± 0.27, $p < 0.05$). The striatal asymmetry index was 18.1 ± 12.5% in SCA2 and 25 ± 15.4% in PD ($p = 0.37$).

Striatal V_3'' correlated with the total ICARS score ($r = -0.836$, $p < 0.05$) but not with the duration of disease ($r = -0.546$, $p = 0.26$) in SCA2 patients.

Discussion

This [^{123}I]FP-CIT SPECT study is the first to assess *in vivo* the status of nigrostriatal dopaminergic projections in patients with SCA2 without parkinsonism and to compare it with PD. A pronounced reduction of striatal DAT density was found in SCA2 patients compared with controls. The degree of whole striatal DAT decrease was similar to that of PD patients with mild-to-moderate disease. The DAT decrease was homogeneous, involving caudate and putamen with a similar degree, and asymmetrical as in PD. Moreover, striatal DAT density in SCA2 patients significantly correlated with the total ICARS.

Evidence of impaired presynaptic dopaminergic function has been demonstrated in a previous case report study of a SCA2 patient with severe, disabling resting and action tremor[17] or in studies of patients with familial L-dopa responsive parkinsonism associated with SCA2 mutation.[11,12,14] In these studies, the imaging findings were a pronounced decrease of striatal [^{123}I]β-CIT,[17] [^{99m}Tc]Trodat-1,[12] or [^{18}F]fluorodopa[11,14] uptake of similar degree as idiopathic PD patients, with more uniform involvement of caudate and putamen in some cases.[11,17] In this study, we found that SCA2 patients without parkinsonian symptoms also show a decrease of striatal DAT density as idiopathic PD. However, the pattern of reduction was different from that found in idiopathic PD in which a prevalent involvement of putamen with respect to caudate is present. The putamen to caudate ratio was the variable that best discriminated SCA2 and PD patients in this study, suggesting a different extent of substantia nigra pathology in the two disorders.

The correlation between [^{123}I]FP-CIT V_3'' and the ICARS is difficult to explain. The ICARS is a scale highly weighted on the severity of cerebellar ataxia including disturbance of posture, gait, and kinetic functions with some contribution from speech and oculomotor disorder.[15] The patients did not have any rest tremor, akinesia, or rigidity and presented with mild to moderate gait and cerebellar kinetic dysfunction. However, the presence of nigrostriatal degeneration could contribute to the severity of the disease explaining the correlation found between DAT density and the ICARS. It may also be possible that cerebellar, pontine, and substantia nigra degeneration follow a similar and parallel course and that the progression of the disease in SCA2 patients occurs similarly in the above regions explaining why a correlation between apparently unrelated parameters was observed.

It is intriguing that SCA2 patients show a similar degree of nigrostriatal dopaminergic dysfunction as that of PD patients, even though after a longer disease duration (10 ± 4 vs 6 ± 3 years, $p = 0.06$), in absence of evident parkinsonism. Parkinsonian signs could be masked by the prevalence of ataxic disturbances also in the presence of significant nigrostriatal impairment, or alternatively the involvement of other nuclei in the extrapyramidal system such as the globus pallidus and the subthalamic nucleus[6,8] could account for the lack of parkinsonism.

This study suggests that [^{123}I]FP-CIT SPECT provides a way to evaluate *in vivo* the degree of nigrostri-

atal dopaminergic dysfunction in SCA2 patients independently of the presence of parkinsonian signs.

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CYP2D6 Polymorphism, Pesticide Exposure, and Parkinson's Disease

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We performed a case–control study of Parkinson's disease (PD) in a population characterized by a high prevalence of pesticide exposure and studied the joint effect of pesticide exposure and CYP2D6. Although they are based on a small group of subjects with the joint exposure, our findings are consistent with a gene–environment interaction disease model according to which (1) pesticides have a modest effect in subjects who are not CYP2D6 poor metabolizers, (2) pesticides' effect is increased in poor metabolizers (approximately twofold), and (3) poor metabolizers are not at increased PD risk in the absence of pesticide exposure.

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Several studies suggest that exposure to pesticides may be associated with Parkinson's disease (PD) among humans.[1–3] Recent laboratory and animal studies support this hypothesis.[4,5] In addition, it has been hypothesized that this association may be stronger among genetically susceptible individuals.[2,3]

The debrisoquine hydroxylase in cytochrome P450 D6 (CYP2D6) metabolizes several xenobiotics, including organophosphate pesticides, the herbicide atrazine, and MPTP, a toxin that induces a parkinsonian syndrome and is structurally close to the herbicide Paraquat.[6–8] CYP2D6 activity is genetically determined. Poor metabolizers (PMs) have undetectable CYP2D6 activity and represent 5 to 10% of whites. This trait is inherited as a recessive autosomal trait, and the most frequent polymorphism among white PMs is a G/A

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