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PET in LRRK2 mutations: comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation

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Parkinson's disease may arise from multiple aetiologies, including genetic mutations that are for the most part uncommon. We describe here the positron emission tomography (PET) findings in clinically affected and asymptomatic, high-risk members of two autosomal dominantly inherited Parkinson's disease kindreds with recently described mutations at the PARK8 locus, in a novel gene encoding a leucine-rich repeat kinase (LRRK2). Affected family members have L-dopa-responsive parkinsonism with loss of dopaminergic nigral neurons and pleomorphic subcellular pathology. Fifteen family members underwent PET using: ^{18}F-6-fluoro-L-dopa (^{18}F-dopa) to assess dopamine (DA) synthesis and storage, ^{11}C-(±)α-dihydrotetrabenazine (^{11}C-DTBZ) for the vesicular monoamine transporter, and ^{11}C-*d-threo*-methylphenidate (^{11}C-MP) for the membrane dopamine transporter (DAT). Measurements were compared with normal (n = 33) and sporadic Parkinson's disease (sPD) (n = 67) control groups. Four clinically affected members had findings similar to sPD, with impaired presynaptic DA function affecting the putamen more than the caudate. In two affected members, D2 dopamine receptor binding was intact. Two asymptomatic mutation carriers had abnormal DAT binding with another two developing such abnormalities over 4 years of follow-up. In these individuals, ^{18}F-dopa uptake remained normal, although two of them also displayed abnormal ^{11}C-DTBZ binding. Our study demonstrates that the *in vivo* neurochemical phenotype of LRRK2 mutations is indistinguishable from that of sPD, despite the pathological heterogeneity of the condition. Furthermore, we suggest that compensatory changes including downregulation of the DAT and upregulation of decarboxylase activity may delay the onset of parkinsonian symptoms.

Keywords: genetics; Parkinson's disease; pathophysiology; positron emission tomography

Abbreviations: ^{11}C-DTBZ = ^{11}C-(±)α-dihydrotetrabenazine; ^{11}C-MP = ^{11}C-*d-threo*-methylphenidate; ^{11}C-RAC = ^{11}C-raclopride; ^{18}F-dopa = ^{18}F-6-fluoro-L-dopa; BP = binding potential; DAT = dopamine transporter; LRRK2 = leucine-rich repeat kinase 2; PET = positron emission tomography; sPD = sporadic Parkinson's disease; UPDRS = Unified Parkinson's Disease Rating Scale; VMAT2 = vesicular monoamine transporter

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Introduction

Parkinson's disease is one of the most common neurodegenerative disorders with a prevalence of 1–2 in 100 for the 65 years and older population (de Rijk *et al.*, 2000). Parkinson's disease is characterized by tremor, rigidity, bradykinesia/akinesia and postural instability, resulting from the loss of dopaminergic neurons within the substantia

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nigra pars compacta (SNc). Although most Parkinson's disease is sporadic, several mutations resulting in parkinsonism have been identified in recent years (Vila and Przedborski, 2004). The clinical expression in some of these disorders differs from that of sporadic Parkinson's disease (sPD) in features such as age of onset, rate of progression, associated neurological features and the incidence of complications. Neuropathological and neurochemical characterization of most of these forms of parkinsonism has been limited, and thus their relationship to sPD has for the most part remained unresolved.

The most recent addition to dominantly-inherited causes of Parkinson's disease involves a gene encoding a newly described large, multifunctional protein, leucine-rich repeat kinase LRRK2 (leucine-rich repeat kinase 2) in which eight mutations have been described (Paisan-Ruiz *et al.*, 2004; Zimprich *et al.*, 2004*a*; Di Fonzo *et al.*, 2005; Kachergus *et al.*, 2005). Gly2019Ser mutations in this gene may account for between 1–2 and 5–7% of sporadic and familial Parkinson's disease, respectively (Aasly *et al.*, 2005; Gilks *et al.*, 2005; Nichols *et al.*, 2005). Patients with mutations in this gene have L-dopa-responsive parkinsonism with typical complications of therapy, but while loss of dopamine-producing neurons of the SNc is seen in all, the ultrastructural pathology is highly pleomorphic, with Lewy bodies seen in some but not others, while some subjects display abnormal tau and even amyloid deposition.

Positron emission tomography (PET), by providing quantitative information on dopaminergic function, is useful for the *in vivo* investigation of Parkinson's disease. ^{18}F-6-fluoro-L-dopa (^{18}F-dopa) uptake correlates with the number of nigral dopamine (DA) neurons in humans (Snow *et al.*, 1993) and in animal models of Parkinson's disease (Pate *et al.*, 1993). PET or SPECT studies with ^{18}F-dopa, ^{11}C-(±)α-dihydrotetrabenazine (^{11}C-DTBZ) or a variety of dopamine transporter (DAT) markers have consistently demonstrated a rostrocaudal gradient of striatal presynaptic DA dysfunction in sPD with the putamen more affected than the caudate (Garnett *et al.*, 1983; Brooks *et al.*, 1990; Frey *et al.*, 1996; Guttman *et al.*, 1997; Lee *et al.*, 2000). Subclinical nigrostriatal DA dysfunction has been previously demonstrated in subjects exposed to MPTP (Calne *et al.*, 1985), and in subjects with a high genetic risk of Parkinson's disease (Piccini *et al.*, 1999).

In the present PET study, we sought to characterize the neurochemical phenotype of clinically affected and asymptomatic, high-risk individuals from two dominantly-inherited Parkinson's disease families with mutations in LRRK2. Presynaptic striatal dopaminergic function was evaluated using ^{18}F-dopa to assess dopa uptake, decarboxylation via L-aromatic amino acid decarboxylase (L-AADC) and storage as ^{18}F-dopamine; whereas, ^{11}C-*d-threo*-methylphenidate (^{11}C-MP) assessed the DAT and ^{11}C-DTBZ provided a more effective marker of nerve terminal integrity due to the reduced susceptibility of vesicular monoamine transporter (VMAT2) to pharmaceutical and compensatory mechanisms (Vander Borght *et al.*, 1995; Kilbourn *et al.*, 1996). In two affected subjects, postsynaptic striatal D2 receptor function was assessed using ^{11}C-raclopride (^{11}C-RAC).

Methods

Study population

Fifteen members from two well-documented families with autosomal dominantly inherited Parkinson's disease (families A and D) were investigated with PET. The Family A (German-Canadian) pedigree consists of 208 members, spanning six generations with at least 15 affected members. Family D (Western Nebraska) consists of 190 members with 22 affected members spanning six generations. Affected members from both families demonstrate L-dopa-responsive parkinsonism with typical therapy-related complications. Amyotrophy, dementia, dystonia, tremor and generalized epilepsy have occurred in some members from Family A. Previously reported PET studies with ^{18}F-dopa in one affected member from each family and ^{11}C-RAC in one affected member from Family A demonstrated findings similar to sPD (Wszolek *et al.*, 1995, 1997). Pathological findings include nigral neuronal loss with gliosis in all. Variable subcellular findings have included diffuse or brainstem-restricted Lewy bodies, non-specific eosinophilic granules, mild anterior horn cell loss, senile plaques and neurofibrillary tangles. One case demonstrated neither tangles nor Lewy bodies (Wszolek *et al.*, 1995, 1997, 2004). Linkage to the PARK 8 locus on chromosome 12 has been demonstrated for both families (Zimprich *et al.*, 2004*b*). In Family A, there is a Y1699C mutation and in family D there is a R1441C substitution in the gene encoding LRRK2 (Zimprich *et al.*, 2004*a*). PET data from 33 healthy volunteers and 67 patients with clinically definite sPD (Calne *et al.*, 1992), obtained with identical imaging and data analysis protocols, were included for comparison. At the time of investigation four family members, all carrying mutations in LRRK2, had clinically definite Parkinson's disease. Of these subjects, one was receiving L-dopa/carbidopa (350 mg/day), another pramipexole (3 mg/day) and a third selegiline (5 mg/day). The fourth subject, diagnosed at the time of study, was receiving no antiparkinsonian therapy. The remaining subjects were asymptomatic. Table 1 summarizes the characteristics of the subjects and controls. As a part of an ongoing follow-up study, several family members have been rescanned using the same protocol. All subjects gave written informed consent. This study was approved by the Clinical Research Ethics Board of the University of British Columbia.

Tracer chemistry

^{11}C-DTBZ was synthesized using a modification of the method of Kilbourn *et al.* (1995). ^{11}C-*d-threo*-MP was synthesized by modification of the procedure of Ding *et al.* (1994). The chemical synthesis for ^{18}F-dopa has been described elsewhere (Adam and Ruth, 1988). ^{18}F was produced as F_2 via the double shoot method making use of the ^{18}O(p,n)^{18}F reaction to produce the radioactivity and a second irradiation using F_2/Ar gas mixture for the recovery of ^{18}F-F_2 (Nickles *et al.*, 1984). The enriched ^{18}O-O_2-target gas was recovered using a system similar to that described in the literature (Ruth *et al.*, 2001). ^{11}C-RAC was synthesized as previously described (Ehrin *et al.*, 1987).

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Table 1 Family A (German-Canadian) and Family D (Western Nebraska) subject and control characteristics at the time of the first scans

	Mutation status*	Clinical status	Age (years)	Sex	UPDRS III
Familial subjects					
1	+	PD†	51	M	30
2	+	PD	44	M	14
3	+	PD‡	77	M	7§
4	+	PD	60	F	6
5	+	A	>	M	0
6	+	A	>	F	5
7	–	A	<	M	1
8	+	A	>	F	4‖
9	+	A	<	F	0
10	–	A	>	F	0
11	–	A	<	F	2
12	+	A	<	F	0
13	–	A	>	F	3
14	+	A	<	F	5
15	N/A¶	A	<	M	1
Normal controls					
N = 33		–	54.76 ± 14.15**	14M/19F	–
sPD controls					
N = 67		PD (8.02 ± 6.17)**††	61.04 ± 9.44**	52M/15F	30.2 ± 11.7**

A, asymptomatic; PD, Parkinson's disease; sPD, sporadic Parkinson's disease control group. *LRRK2 mutation, †disease duration 1 year or less for Subjects 1–4, ‡Subject 3 diagnosed at the time of the PET scan, §Subject 3 had prior foot surgery, ‖symptoms due to sequelae of poliomyelitis, ¶N/A = not available, this person elected not to provide blood sample for the genetic analysis, genealogically at risk, **mean ± SD, ††mean disease duration in years. < = age < 55 years; > = age > 55 years. Ages of at risk individuals are not shown in order to protect anonymity.

PET studies and image analysis

All antiparkinsonian medications were stopped at least 12 h before each assessment. (18 h for controlled release L-dopa/carbidopa and dopamine agonists). Subjects fasted overnight and received a standard low-protein breakfast the morning of scanning. Subjects underwent brief clinical examination including videotaped motor Unified Parkinson's Disease Rating Scale (UPDRS) (Fahn *et al.*, 1987). PET scans were performed consecutively in the order of ^{11}C-DTBZ, ^{11}C-MP and ^{18}F-dopa, in a single day for most cases. If included, ^{11}C-RAC scans were performed the following day. All scans were performed in three-dimensional mode with an ECAT 953B/31 tomograph (CTI/Siemens, Knoxville, TN). Data processing and reconstruction are described in detail elsewhere (Sossi *et al.*, 1998). Subjects were positioned supine with the gantry parallel to the orbitomeatal line and the head centred in the field of view. A thermoplastic mask was used to minimize movement and for repositioning in subsequent scans.

A transmission scan with ^{68}Ge rods for attenuation correction was obtained ~10 min prior to injection of each radioligand. Using a Harvard infusion pump, ^{11}C-DTBZ (185 MBq in 10 ml of saline) was injected intravenously over 60 s. A series of sequential emission scans was obtained (4 × 1-min, 3 × 2-min, 8 × 5-min, 1 × 10-min) starting at tracer injection, for a total acquisition time of 60 min. Following an interval of 2.5 h (i.e. >7 half-lives for ^{11}C) to allow for radioactive decay, subjects were re-positioned and ^{11}C-MP (185 MBq in 10 ml of saline) was injected. Scans were acquired over 60 min as above. Following an additional interval of at least 2.5 h subjects received ^{18}F-dopa (185–260 MBq in 10 ml of saline). One hour prior to ^{18}F-dopa injection subjects received 200 mg of carbidopa orally. Nine sequential emission scans, each lasting 10 min, were obtained over 90 min. For subjects receiving ^{11}C-RAC, 185 MBq in 10 ml of saline were injected and data were acquired as for ^{11}C-DTBZ and ^{11}C-MP.

The methods of image analysis have been described in detail elsewhere (Lee *et al.*, 2000). In brief, for all tracers regions of interest (ROIs) were placed on the summed images from the last 30 min of scanning in the five adjacent slices which best demonstrated the striatum. One circular ROI (area 61.2 mm^2, diameter 8.8 mm) was placed on each caudate head. Three additional ROIs of identical dimension were placed sequentially along the rostrocaudal axis of each putamen without overlap. For ^{18}F-dopa, ^{11}C-DTBZ and ^{11}C-MP larger, circular ROIs (area 297 mm^2, diameter 19.4 mm) were positioned 3 per side over the cortex of the temporo-occipital lobe. For ^{11}C-RAC, a single large ROI (area 2107 mm^2) was placed over the cerebellum. The ROIs were then replicated on each acquired time frame to obtain a time activity curve for each sampled region.

Binding potentials (BP) for ^{11}C-MP, ^{11}C-DTBZ and ^{11}C-RAC data were obtained using a multiple time graphical method (Logan *et al.*, 1996) with an occipital lobe input function (cerebellar for ^{11}C-RAC). ^{18}F-dopa uptake rate constant (K_{occ}) was obtained using a graphical method for unidirectional transport (Patlak and Blasberg, 1985; Martin *et al.*, 1989) with an occipital cortex input function.

Statistical analysis

Left and right mean putaminal K_{occ} and BP values were obtained by averaging the three putaminal ROIs. Mean caudate and putaminal values (K_{occ} or BP) were obtained by averaging the corresponding left and right values. Using simple and multiple regression techniques for each ligand, it was determined that correction was required for age

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when comparing mean caudate or putaminal MP BP values to the normal control group and for age and disease duration (symptom onset to time of imaging) when comparisons of mean caudate MP BP values were made to the sPD control group. For each subject, results were determined as percentile values of the normal or sPD groups with significance derived from the corresponding confidence intervals. The results were ultimately expressed as percent values of normal controls. Statistical significance was set at $P < 0.05$.

Results

To protect the anonymity of the subjects, members from both families have been grouped together and labelled as subjects 1–15. Four subjects demonstrated clinically definite Parkinson's disease (subjects 1–4) with symptom duration $\leq$1 year and UPDRS motor scores ranging from 6 to 30 (Table 1). The remaining subjects were asymptomatic at the time of scanning; those with abnormal PET scans are listed after the clinically affected subjects with the remainder in random order.

The mean age of normal and sPD controls was 54.76 ± 14.15 and 61.04 ± 9.44 years, respectively. Mean symptom duration for the sPD group was 8.02 ± 6.17 years with a mean UPDRS motor score of 30.2 ± 11.7 (Table 1). The mean age for all mutation carriers was 48.9 ± 19.6 years, 58.0 ± 14.3 for those with clinical disease and 42.3 ± 21.3 years for those who remained asymptomatic. The mean age of non-mutation carriers was 59.0 ± 13.8 years. Previous reports have documented mean age of symptom onset at 53 and 65 years for Family A and D, respectively (Wszolek *et al.*, 1997, 2004). In the current analysis, symptom onset was at a mean age of 46.5 ± 4.9 ($n = 2$) and 67.5 ± 12.0 ($n = 2$) years for affected Family A and D members, respectively. PET measurements for all subjects as well as normal and sPD controls are summarized in Table 2. For subjects with abnormal scans, individual ROI values are presented in Table 4. For each ligand, comparison to the normal control group is presented as a percentage (Table 3) with ^{11}C-MP binding potential values corrected for age. LRRK2 gene mutation status is presented in Tables 1–3.

^{18}F-dopa

The mean putaminal uptake constant (K_{occ}) for ^{18}F-dopa was 0.0104 ± 0.0011 min^{-1} and 0.0046 ± 0.0017 min^{-1} for the normal and sPD control groups, respectively. For each of the clinically affected subjects, ^{18}F-dopa uptake was significantly reduced when compared to the normal control group and demonstrated asymmetry and a rostrocaudal gradient of severity, similar to that reported in sPD (Garnett *et al.*, 1983; Martin *et al.*, 1989; Brooks *et al.*, 1990), with the putamen more severely affected (Table 4). There was no significant difference between putaminal values of affected family members and sPD controls. Subject 11 was unable to complete the ^{18}F-dopa component of the protocol.

^{11}C-DTBZ

The mean putaminal BP for ^{11}C-DTBZ was 0.98 ± 0.009 and 0.35 ± 0.15 for the normal and sPD control groups, respectively. Significantly reduced BP values for ^{11}C-DTBZ were demonstrated for all clinically affected subjects when compared to normal controls. As for ^{18}F-dopa uptake, asymmetry and a rostrocaudal gradient were observed (Table 4). Subject 1 did not complete either the ^{11}C-DTBZ or ^{11}C-MP protocol. No difference was detected between clinically affected subjects and sPD controls. One asymptomatic mutation carrier (Subject 5) demonstrated significantly reduced ^{11}C-DTBZ BP (54% of normal) when compared to normal controls. For this individual, ^{18}F-dopa uptake remained within normal limits, whereas age-adjusted ^{11}C-MP binding was also reduced to 51% of normal.

^{11}C-MP

The mean putaminal BP for ^{11}C-MP was 1.32 ± 0.002 and 0.41 ± 0.20 (0.49 ± 0.24, age-adjusted) for the normal and sPD control groups, respectively. As for ^{18}F-dopa K_{occ} and ^{11}C-DTBZ BP, age-adjusted values for ^{11}C-MP BP in clinically affected members were significantly reduced compared to normal, with asymmetry and a rostrocaudal gradient (Table 4). In addition, two asymptomatic subjects (Subjects 5 and 6, both mutation carriers) also demonstrated significant reductions in putaminal age-adjusted ^{11}C-MP BP. As noted above, Subject 5 demonstrated reduced putaminal values for ^{11}C-DTBZ binding as well as age-adjusted ^{11}C-MP binding, but ^{18}F-dopa uptake was normal. Whereas Subject 6 demonstrated significantly reduced age-adjusted ^{11}C-MP binding at 71% of normal, ^{18}F-dopa and ^{11}C-DTBZ values for this individual were normal. Adjusted ^{11}C-MP binding in clinically affected members and asymptomatic Subject 5 was not significantly different from the sPD comparison group, but the putaminal age-adjusted ^{11}C-MP BP for asymptomatic Subject 6 was significantly increased compared to sPD controls.

^{11}C-RAC

^{11}C-RAC PET was performed on two clinically affected family members (Subjects 1 and 4). BP values for both subjects were within the range of normal and consistent with sPD (2.34 and 2.23 for putamen and 2.71 and 2.19 for caudate, respectively).

Follow-up scans

As part of an ongoing study, returning subjects underwent repeat scanning, using an identical protocol, on average 4 years after the original scans. Of those returning to date, two asymptomatic mutation carriers (Subjects 12 and 14) with previously normal scans had abnormalities. One demonstrated significantly reduced ^{11}C-DTBZ binding (putamen only) at 81% of normal and age-adjusted ^{11}C-MP binding at 69% of normal. These findings represent reductions of ~13 and 14% (age-adjusted) from original scan results,

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Table 2 Striatal PET measurements (first scans) for familial subjects and controls*

	Mutation status†	^{18}F-dopa‡		^{11}C-DTBZ§		Age-adjusted ^{11}C-MP‖	
		Caudate	Putamen	Caudate	Putamen	Caudate	Putamen
Familial subjects¶							
1**	+	0.00905††	0.00564††	–	–	–	–
2**	+	0.00868††	0.00500††	0.60848††	0.38648††	0.54554††	0.23284††
3**	+	0.01081	0.00762††	0.65498††	0.50066††	1.27505	0.82045††
4**	+	0.00922††	0.00589††	0.50170††	0.40475††	0.79312††	0.51671††
5	+	0.01305	0.00934	0.72220††	0.52603††	1.06713††	0.67402††
6	+	0.01264	0.01047	1.00000	0.85000	1.47614	0.93502††
7	–	0.01074	0.00931	1.06549	1.11770	1.70557††	1.59258
8	+	0.01099	0.00936	0.89030	0.86342	1.35062	1.10361
9	+	0.01105	0.00993	1.03959	0.95320	1.38387	1.06290
10	–	0.01272	0.01111	0.94007	0.92108	1.22997	1.19937
11	–	–	–	1.10500	1.06500	1.53164	1.41302
12	+	0.00960	0.00916	1.02651	1.05097	1.29095	1.20095
13	–	0.01295	0.01238	0.98574	0.95835	1.50285	1.32697
14	+	0.01118	0.01084	0.88943	0.92472	1.20829	1.09258
15	N/A	0.01004	0.00910	1.02400	0.83904	1.59396	1.13833
Normal controls							
$N = 33$		0.01158 ± 0.00103#	0.01039 ± 0.00111	0.96723 ± 0.00083	0.97940 ± 0.00883	1.45975 ± 0.00229§§	1.31867 ± 0.00235§§
sPD controls							
$N = 67$		0.00853 ± 0.00168#	0.00460 ± 0.00170	0.53924 ± 0.15855	0.35256 ± 0.15115	0.84752 ± 0.25208	0.49013 ± 0.23569

PET, positron emission tomography; sPD, sporadic Parkinson's disease control group; ^{18}F-dopa, ^{18}F-6-fluoro-L-dopa; ^{11}C-DTBZ, ^{11}C-(±)α-dihydrotetrabenazine; ^{11}C-MP, ^{11}C-*d-threo*-methylphenidate. *Averaged PET measurements for left and right sides, †LRRK2 mutation, ‡K_{occ} (min^{-1}), §binding potential (B_{max}/K_d), ‖age-adjusted binding potential (B_{max}/K_d), ¶clinically affected and individuals with abnormal PET scans listed first with the remainder randomly listed to protect anonymity, ††PET measurements significantly different to normal control group, $P < 0.05$, **symptomatic subjects, #mean ± SD, §§unadjusted binding potential (B_{max}/K_d).

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Table 3 Percent values: comparison to normal controls for putamen*

	Mutation status†	^{18}F-dopa (% normal)	^{11}C-DTBZ (% normal)	Adjusted ^{11}C-MP‡ (% normal)
sPD Controls§				
N = 67		44.3 (11.8, 80.9)	36.0 (5.3, 69.0)	37.2 (1.7, 76.1)
Familial subjects				
1‖	+	54.3¶	–	–
2‖	+	48.1¶	39.5¶	17.7¶
3‖	+	73.4¶	51.1¶	62.2¶
4‖	+	56.7¶	41.3¶	39.2¶
5	+	89.9	53.7¶	51.1¶
6	+	95.2	86.8	70.9¶
7	–	89.7	114.1	120.8
8	+	90.1	88.2	83.7
9	+	95.6	97.3	80.6
10	–	107.0	94.1	91.0
11	–	–	108.7	107.2
12	+	88.2	107.3	91.1
13	–	119.2	97.9	100.6
14	+	104.4	94.4	82.9
15	N/A	87.6	85.7	86.3

sPD, sporadic Parkinson's disease control group; ^{18}F-dopa, ^{18}F-6-fluoro-L-dopa; ^{11}C-DTBZ, ^{11}C-(±)α-dihydrotetrabenazine; ^{11}C-MP, ^{11}C-*d-threo*-methylphenidate. *Percent (%) values for putamen in subjects relative to normal control group, †LRRK2 mutation, ‡MP values for subjects corrected for age when compared to normal control group, §mean (95% CIs), ‖symptomatic subjects, ¶percent values significantly different to normal control group, $P < 0.05$.

respectively. ^{18}F-dopa uptake remained normal with only a 2.4% reduction. A second asymptomatic member demonstrated significantly reduced age-adjusted ^{11}C-MP at 76% of normal, a reduction of ~15% (age-adjusted) from the original scan. Reductions of ~5% and 12% were seen for ^{18}F-dopa uptake and ^{11}C-DTBZ binding, respectively, but levels were still within normal limits. At follow-up, both subjects were a minimum of 10 years younger than the average age of onset of parkinsonism in their respective families and motor examination revealed no evidence of parkinsonism (motor UPDRS scores of 1 and 2, respectively).

Discussion

We have shown that in affected members from two families with autosomal dominantly inherited parkinsonism linked to PARK8, the neurochemical profile of dopaminergic dysfunction as assessed by PET is indistinguishable from that of sPD. Thus, affected individuals have reductions in ^{18}F-dopa uptake and in binding of ^{11}C-MP and ^{11}C-DTBZ to the DAT and VMAT2, respectively, while in both individuals so studied, post-synaptic dopamine D2 receptors were intact. Our findings are consistent with recent studies of ^{18}F-dopa PET in six patients with a Gly2019Ser mutation of LRRK2 (Hernandez *et al.*, 2005) and one additional subject with linkage to the PARK8 locus (Paisan-Ruiz *et al.*, 2005). The pattern of DA dysfunction is typical of that seen in sPD, with relative sparing of the caudate nucleus and more severe impairment of the putamen. Affected subjects from both families showed asymmetric reduction of tracer uptake typical of sPD, and in contrast to some forms of inherited parkinsonism, PET values were in keeping with clinical severity when compared to sPD.

The families described here have dominantly inherited, L-dopa-responsive parkinsonism associated with typical complications of long-term treatment. In both families, neurological disease arises from mutations in a newly described gene, designated LRRK2. LRRK2 is a member of the recently defined ROCO family of proteins that have five conserved domains, including a leucine-rich repeat. The fact that at least eight mutations in LRRK2 have been described in numerous families suggests that mutations in this gene are a much more common cause of inherited Parkinson's disease than other dominantly inherited mutations described to date and may account for up to 7% of familial Parkinson's disease and between 1 and 2% of sPD (Aasly *et al.*, 2005; Gilks *et al.*, 2005; Nichols *et al.*, 2005). Importantly, the pathology described in seven individuals from these two families is heterogeneous. While all affected members have shown loss of DA neurons in the SNc and associated gliosis, only one individual from Family D had Lewy bodies restricted to the brainstem, and another had diffuse Lewy bodies. Three individuals from both kindreds showed neurofibrillary tangles and abnormal tau deposits, with senile plaques seen in three other individuals from both families. In affected members of Family A, there is additionally anterior horn cell loss associated with axonal spheroids.

This striking heterogeneity raises issues of fundamental importance. First, it is clear that multiple pathological expressions can arise from the same disease. Indeed, all of the described subjects had Parkinson's disease based on clinical criteria, even though only a single individual showed typical Lewy body pathology restricted to the substantia nigra. Other causes for Parkinson's disease include conditions in which there is nigral cell loss in the absence of Lewy bodies

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Table 4 PET values of individual ROIs for subjects with abnormal PET findings

Subject	ROI	^{18}F-dopa*		^{11}C-DTBZ†		Age-adjusted ^{11}C-MP‡	
		Right	Left	Right	Left	Right	Left
1§	Caud	0.00963	0.00846	–	–	–	–
	Put1	0.00712	0.00797	–	–	–	–
	Put2	0.00585	0.00474	–	–	–	–
	Put3	0.00495	0.00325	–	–	–	–
2§	Caud	0.00872	0.00863	0.65212	0.56483	0.51521	0.57588
	Put1	0.00700	0.00673	0.47754	0.52007	0.39770	0.35373
	Put2	0.00463	0.00499	0.35868	0.35137	0.23859	0.22874
	Put3	0.00349	0.00316	0.29925	0.31196	0.13226	0.04608
3§	Caud	0.01117	0.01045	0.68570	0.62425	1.30087	1.24923
	Put1	0.00947	0.00924	0.62735	0.65477	1.03585	1.08301
	Put2	0.00710	0.00834	0.53423	0.58040	0.74115	0.89534
	Put3	0.00467	0.00691	0.26689	0.34029	0.54545	0.62192
4§	Caud	0.00822	0.01022	0.47764	0.52576	0.85290	0.73334
	Put1	0.00644	0.00746	0.44005	0.46096	0.65831	0.66365
	Put2	0.00523	0.00567	0.39508	0.40498	0.43214	0.50830
	Put3	0.00508	0.00546	0.34007	0.38737	0.39199	0.44592
5	Caud	0.01276	0.01336	0.72966	0.71474	1.06886	1.06541
	Put1	0.01043	0.01150	0.68445	0.66775	0.87263	0.93814
	Put2	0.00992	0.00889	0.60774	0.50792	0.63927	0.67500
	Put3	0.00690	0.00838	0.33308	0.35523	0.40586	0.51322
6	Caud	0.01260	0.01268	1.05991	0.94210	1.58282	1.37210
	Put1	0.01159	0.01220	1.02288	0.98137	1.42477	1.21003
	Put2	0.00909	0.00971	0.92387	0.80157	1.02338	0.81531
	Put3	0.00757	0.00915	0.69862	0.66906	0.62800	0.56020
12‖	Caud	0.01054	0.01068	0.98811	1.03362	1.22911	1.33722
	Put1	0.00973	0.00919	1.01301	1.01123	1.33663	1.23742
	Put2	0.00887	0.00865	0.93161	1.01993	1.13057	0.99317
	Put3	0.00742	0.00770	0.87608	0.77072	0.73345	0.61373
14‖	Caud	0.01125	0.01196	0.84167	0.88434	1.05014	1.18000
	Put1	0.01137	0.01154	0.89247	0.88845	1.10817	1.11155
	Put2	0.01046	0.01100	0.82108	0.92717	0.87781	0.93759
	Put3	0.00978	0.00939	0.57619	0.67809	0.68700	0.73163
Normal** controls (N = 33)	Caud	0.01164 ± 0.00108††		0.97048 ± 0.08714		1.46094 ± 0.21856	
	Put1	0.01166 ± 0.00120		1.05093 ± 0.11265		1.50265 ± 0.22141	
	Put2	0.01093 ± 0.00123		1.06068 ± 0.10105		1.37768 ± 0.24554	
	Put3	0.00882 ± 0.00131		0.83038 ± 0.11279		1.06585 ± 0.24326	
sPD controls (N = 67)	Caud	0.00853 ± 0.00168		0.53924 ± 0.15855		0.84752 ± 0.25208	
	Put1	0.00685 ± 0.00195		0.48040 ± 0.16633		0.68627 ± 0.26776	
	Put2	0.00417 ± 0.00190		0.34775 ± 0.16420		0.45990 ± 0.23644	
	Put3	0.00278 ± 0.00154		0.22954 ± 0.14333		0.32062 ± 0.22072	

ROI, region of interest; ^{18}F-dopa, ^{18}F-6-fluoro-L-dopa; ^{11}C-DTBZ, ^{11}C-(±)α-dihydrotetrabenazine; ^{11}C-MP, ^{11}C-*d-threo*-methylphenidate; Caud, caudate; Put1, anterior putamen; Put2, mid putamen; Put3, posterior putamen; sPD, sporadic Parkinson's disease control group. *K_{occ} (min^{-1}), †binding potential (B_{max}/K_d), ‡age-adjusted binding potential (B_{max}/K_d) except for normal controls, §symptomatic subjects, ‖repeat scans after 4 years of follow-up, **mean values for left and right caudate and anterior, mid and posterior putamen, ††mean ± SD.

(e.g. parkin mutations), whereas nigral Lewy bodies have also been described in other neurodegenerative conditions (e.g. Hallervorden Spatz disease) (Arawaka *et al.*, 1998) and in the absence of parkinsonism (Fearnley and Lees, 1991). Thus, the demonstration of PET findings typical of sPD in PARK 8 parkinsonism supports the view that the single most important pathological feature shared by the various forms of Parkinson's disease is the regional distribution of nigral neuronal loss, rather than the subcellular changes, which, as seen in this disorder, can be highly variable despite a single, well-defined aetiology. Other less common causes of inherited Parkinson's disease such as mutations in the gene encoding α-synuclein also result in PET profiles typical of sPD (Samii *et al.*, 1999), although that condition has a clinical course which is somewhat more aggressive than typical Parkinson's disease (Golbe *et al.*, 1990). In contrast, in subjects with parkin-related parkinsonism, presynaptic DA dysfunction may range from mild to severe, with the caudate and putamen similarly affected, or a rostrocaudal gradient may be seen as in sPD (Broussolle *et al.*, 2000; Hilker *et al.*, 2001; Portman *et al.*, 2001; Scherfler *et al.*, 2004). Furthermore, post-synaptic dysfunction in the form of reduced D2 receptor binding has been reported (Hilker *et al.*, 2001; Scherfler *et al.*, 2004). Similarly, patients with PARK 6 parkinsonism also

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display more severe involvement of the anterior striatum than do patients with sPD (Khan *et al.*, 2002).

Four of the asymptomatic mutation carriers reported here had reduced DAT binding. In two, DTBZ binding was significantly lower than normal, but reduced to a lesser degree than symptomatic sPD, suggesting very early nerve terminal loss. In all of the asymptomatic mutation carriers, ^{18}F-dopa uptake was normal, despite the other evidence for impaired DA function, suggesting that L-AADC activity in these subjects was sufficient to maintain DA levels and avoid symptoms. Taken together, these findings are in keeping with other evidence for differential involvement of L-AADC, VMAT2 and DAT in Parkinson's disease (Wilson *et al.*, 1996; Lee *et al.*, 2000), possibly reflecting compensatory changes that serve to maintain extracellular levels of DA.

In summary, clinically affected and unaffected mutation carriers of these families demonstrate PET changes indistinguishable from those of sPD, in which there is dysfunction of presynaptic DA function affecting the putamen more than the caudate nucleus. The greater reduction of DAT than changes in VMAT2 and L-AADC in preclinical disease is in keeping with compensatory mechanisms that have been described in sPD, but could alternatively reflect a disease process that preferentially targets the DAT in early disease. The ability to reliably detect nigrostriatal dysfunction at an early preclinical stage may ultimately allow the use of neuroprotective therapies designed to halt or slow disease progress prior to symptom development in subjects at high risk.

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