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Clinical and Subclinical Dopaminergic Dysfunction in PARK6-Linked Parkinsonism: An ^{18}F -dopa PET Study

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PARK6, a locus for early-onset recessive parkinsonism, has been causally implicated in nine unrelated families from four different countries. The gene is still unidentified and hence the importance of PARK6 as a cause of Parkinson's disease is unknown. To date, no pathology or functional imaging studies are available on PARK6-linked parkinsonism. We have used ^{18}F -dopa positron emission tomography to study four patients who are homozygous and three asymptomatic relatives who are heterozygous for PARK6. The clinically affected PARK6 subjects had a similar 85% reduction in posterior dorsal putamen ^{18}F -dopa uptake to a group of idiopathic Parkinson's disease patients matched for clinical disease severity and duration but showed significantly greater involvement of head of caudate and anterior putamen. The group of asymptomatic PARK6 carriers showed a significant mean 20 to 30% reduction in caudate and putamen ^{18}F -dopa uptake in comparison with controls, individual values falling toward the bottom of the normal range. Our results indicate that PARK6 pathology results in a more uniform loss of striatal dopamine terminal function than Parkinson's disease. The subclinical loss of striatal dopamine storage capacity found in the PARK6 carriers implies that the unidentified gene on the short arm of chromosome 1 exhibits either haploinsufficiency or a dominant negative effect.

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A new locus for autosomal recessive parkinsonism was linked to chromosome 1p35-p36, PARK6, in a family from Sicily, the Marsala kindred,¹ and recently has been causally related in a further eight, unrelated European families.² The gene and its disease-causing mutations remain unidentified. Although the importance of PARK6 as a cause of Parkinson's disease is unknown, there is an overlap with the clinical phenotype of idiopathic Parkinson's disease (PD): asymmetrical presentation with unilateral tremor and akinesia in an upper limb and age of onset up to 68 years. Signs such as dystonia and sleep benefit often reported in autosomal recessive juvenile parkinsonism are absent in PARK6.¹ To date, neither postmortem data nor in vivo studies on the function of the nigrostriatal dopaminergic system are available for PARK6-linked parkinsonism.

We have used ¹⁸F-dopa positron emission tomography to study two families: the Marsala kindred (Family 1) and an unrelated Italian family, the Abruzzo kindred (Family 2), in whom disease subsequently was linked to PARK6.^{2,3} The aims were to study the pattern of nigrostriatal dysfunction in PARK6-linked parkinsonism and to assess whether subclinical nigrostriatal dysfunction is present in PARK6 heterozygotes.

Subjects and Methods

Clinically Affected Patients with PARK6-Linked Parkinsonism

Two of the patients with PARK6-linked parkinsonism (one man and one woman) were from the Marsala kindred, Family 1, which contained four affected individuals out of ascertained 122 members¹ (VI.7 and VI.23; Fig 1¹). The other two patients (one man and one woman) were from the

Abruzzo kindred, Family 2, which included 43 family members³ (IV.7 and IV.8, Fig 2³).

Asymptomatic PARK6 Heterozygotes

Three subjects were carriers of a single mutant PARK6 haplotype: two were from Family 1¹ (VI.24, VII.8; see figure 1¹) and one subject was from Family 2³ (III.6; see figure 2³). None of the carriers reported any symptoms or showed any signs of PD.

All subjects underwent a standardized neurological examination. Scores in a practically defined "off" state on the Unified Parkinson's Disease Rating Scale (UPDRS)⁴ and the Hoehn and Yahr (H&Y) scale⁵ were used to rate the degree of parkinsonian disability. All subjects gave informed consent on both occasions, and the project was approved by the ethics committees of the National Hospital for Neurology and Neurosurgery and the Hammersmith Hospitals Trust, London, UK. Permission to administer radiation was licensed by the Administration of Radioactive Substances Advisory Committee UK. The clinical characteristics of all subjects are summarized in Tables 1 and 2.

Scanning Protocol

All subjects were scanned on an ECAT966 scanner (CTI/Siemens, Knoxville, TN) with a reconstructed resolution of 4mm and an axial field of view of 24cm. A dose of approximately 130MBq of ¹⁸F-dopa was administered intravenously over 30 seconds. Scanning began at the start of tracer infusion with 25 time frames over 95 minutes. Before emission data acquisition, a transmission scan was performed with an external rotating positron source of ¹³⁷Cs to allow a measured attenuation correction to be performed. ¹⁸F-dopa positron emission tomography scans were analyzed using a standard region of interest (ROI) approach and multiple time graphical analysis with an occipital reference tissue input function as previously described.^{6,7}

Four ROIs were defined: the head of caudate nucleus with a circular region diameter 10mm, the anterior part of the dorsal putamen with an elliptical region 10 × 12mm, the posterior part of the dorsal putamen with an elliptical region 10 × 12mm, and the entire dorsal putamen with an elliptical region 10 × 24mm aligned along its axis. All ROIs were placed by inspection with reference to the stereotactic atlas of Talairach and Tournoux⁸ on five contiguous transaxial slices. For each patient, we computed head of caudate, and anterior, posterior, and entire putamen ¹⁸F-dopa influx rate constants (Ki) using linear graphical Patlak analysis with an occipital reference tissue input function.⁶ The caudate to putamen ratio of each subject was calculated using the formula $r = \text{mean caudate Ki} / \text{mean putamen Ki}$. The ROI analysis was performed blinded to the genetic status of both the symptomatic subjects (PARK6-linked parkinsonism and PD) and the asymptomatic subjects (PARK6 heterozygotes and control subjects) but not to the clinical status of these two groups.

Eight patients with idiopathic PD matched for disease severity as assessed by UPDRS motor score when withdrawn from medication for 12 hours and Hoehn and Yahr staging (see Table 1) and 14 unrelated, age- and gender-matched controls (mean age, 54.6 ± 13.9; range, 30–71 years; see

Fig. Mean caudate, anterior, and posterior putamen ¹⁸F-dopa uptake in the group of patients with PARK6-linked parkinsonism and in the group of Parkinson's disease patients matched for disease severity. Values are expressed as Ki min⁻¹. (asterisk) p = 0.01; (section mark) p = 0.03.

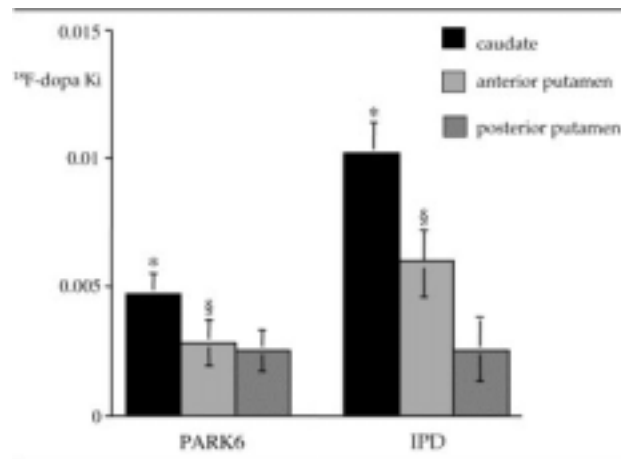


Table 1. Clinical Characteristics and ^{18}F -Dopa Striatal K_i Values in the Four PARK6 Patients and in the Group of Eight Patients with Parkinson's Disease

Patients	Clinical Characteristics					^{18}F -Dopa K_i (min^{-1})				
	Age of Onset (yr)	Age at Scan	DD (yr)	H&Y	UPDRS Motor Score	Caudate	Putamen	Anterior Putamen	Posterior Putamen	C/P Index
PARK6										
VI.7 ^a	45	76	31	3.5	73	0.0051	0.0025	0.0026	0.0025	2.3
VI.23 ^a	38	47	9	2	22	0.0059	0.0022	0.0025	0.0014	1.5
IV.8 ^b	38	41	3	1	5	0.0041	0.0031	0.0029	0.0029	1.2
IV.7 ^b	32	43	11	2	32	0.0043	0.0029	0.0033	0.0028	1.5
Mean	38.2	51.7	13.5	2.1	33.0	0.0047 ^c	0.0027	0.0028 ^d	0.0024	1.8 ^e
($\pm$ SD)	(5.3)	(16.3)	(12.1)	(1.0)	(28.9)	(0.0008)	(0.0004)	(0.0005)	(0.0007)	(0.4)
PD										
Mean	53.0 ^f	67.3 ^g	11.0	3.3	38.4	0.0102	0.0042	0.0060	0.0026	2.8
($\pm$ SD)	(4.5)	(5.5)	(5.1)	(0.7)	(15.0)	(0.0016)	(0.0019)	(0.0015)	(0.0014)	(0.6)

Wilcoxon test (PARK6 patient group vs PD group):

^aFamily 1 (see Fig 1¹).

^bFamily 2 (see Fig 2³).

^c $p = 0.01$;

^d $p = 0.03$;

^e $p = 0.03$;

^f $p < 0.001$;

^g $p = 0.04$.

DD = disease duration; H & Y = Hoehn and Yahr staging⁵; UPDRS = Unified Parkinson's Disease Rating Scale⁵; C/P = caudate/putamen; SD = Standard deviation; PD = Parkinson's disease.

Table 2) were scanned with a similar protocol to the PARK6 subjects. All normal controls reported no family history of parkinsonism and had a normal neurological examination.

Statistical Analysis

The nonparametric Wilcoxon test was used for all comparisons between different groups of patients and controls.

Results

PARK6-Linked Parkinsonism

CLINICAL FINDINGS. These cases all fulfilled the clinical diagnostic criteria of the UK Parkinson's Disease Society Brain Bank for probable PD except for the presence of a family history.⁹ H&Y and UPDRS motor

Table 2. ^{18}F -Dopa Striatal K_i Values in the Three PARK6 Carriers and in the Group of 14 Normal Volunteers

Subjects	Age (yr)	¹⁸ F-Dopa Ki (min ⁻¹)				C/P index
		Caudate	Putamen			
			Putamen	Anterior Putamen	Posterior Putamen	
PARK 6 carriers						
VII.8 ^a	41	0.0099	0.0111	0.0115	0.0102	0.90
VI.24 ^a	49	0.0126	0.0113	0.0131	0.0101	1.10
III.6 ^b	60	0.0131	0.0107	0.0123	0.0105	1.20
Mean	50	0.0119 ^c	0.0110 ^d	0.0123 ^e	0.0102 ^f	1.06
(± SD)	(9.5)	(0.0017)	(0.0003)	(0.0008)	(0.0003)	(0.15)
Controls (n = 14)						
Mean	54.6	0.0153	0.0168	0.0171	0.0163	0.90
(± SD)	(13.9)	(0.0026)	(0.0031)	(0.0029)	(0.0039)	(0.06)

Wilcoxon test (PARK6 carrier group vs controls):

^aFamily 1 (Fig 1¹).

^bFamily 2 (Fig 2³).

^c $p = 0.03$;

^d $p = 0.01$;

^e $p = 0.01$;

^f $p = 0.05$.

C/P index = caudate/putamen index; SD = standard deviation.

scores for each subject at the time of the scan are summarized in Table 1. Mean age of onset and age at scan in the PARK6 group were significantly younger than the PD group ($p < 0.001$; $p = 0.04$, respectively); however, there were no significant differences between the two groups with regard to disease duration and disease severity as rated with the H&Y scale and UPDRS motor scores (see Table 1).

POSITRON EMISSION TOMOGRAPHY FINDINGS. Regional mean ^{18}F -dopa Ki values obtained for the four clinically affected PARK6 patients and the PD group are shown in Table 1. The reductions in posterior putamen ^{18}F -dopa uptake were similarly severe (15% of normal) in the PARK6 patients and the group of PD patients; however, anterior putamen and caudate ^{18}F -dopa uptake was twice in the PD compared with the PARK6 group ($p = 0.03$ and $p = 0.01$, respectively; see Table 1, Fig 1). In addition, the mean caudate to putamen ratio in PARK6 patients was significantly lower compared with the PD group ($p = 0.02$).

Asymptomatic PARK6 Heterozygotes

POSITRON EMISSION TOMOGRAPHY FINDINGS. Individually, all three PARK6 carriers had low normal putamen Ki (>1.5 standard deviation below the normal mean), and one of them (VII.8, see Fig 1¹) also had a low normal caudate Ki (>1.5 standard deviation below the normal mean). As a group, the three carriers had mean caudate and putamen ^{18}F -dopa uptake significantly reduced in comparison with the normal group mean ($p = 0.03$, $p = 0.01$ for caudate and putamen, respectively; see Table 2).

Discussion

This is the first in vivo study of dopamine terminal function in PARK6-linked parkinsonism and asymptomatic carriers of a single PARK6-linked haplotype. We have found that although our groups of PARK6 and idiopathic PD patients were matched for disease duration and clinical disease severity they showed a different pattern of nigrostriatal dopaminergic dysfunction. PARK6 patients had a similar severe reduction in posterior dorsal putamen ^{18}F -dopa Ki to the PD cases but showed twice the involvement of head of caudate and anterior dorsal putamen, which were relatively spared in PD. This resulted in an absence of an anteroposterior gradient of putamen tracer distribution in PARK6. Such a gradient is typical of idiopathic PD and is caused by the preferential degeneration of the ventrolateral tier of the substantia nigra pars compacta, which projects to the posterior dorsal putamen, and relatively sparing of the dorsomedial nigral cells, which project to anterior dorsal putamen and head of caudate.¹⁰ Our data therefore suggest that the neurodegen-

erative process in PARK6-linked parkinsonism involves the nigra more uniformly and may well have different neuropathological features from PD.

Individual positron emission tomography findings of the PARK6 patients are of interest. First, Patient IV.8, with only 3 years of clinical disease, had putamen ^{18}F -dopa values similar to that of his relative IV.7 with 11 years symptom duration and to the idiopathic PD group whose mean disease duration was also 11 years. Second, we observed a disparity between the mild degree of locomotor impairment in Patients VI.23, IV.7, IV.8 and their severe reduction of striatal ^{18}F -dopa uptake. The disparity between moderate clinical impairment and severe nigrostriatal dopaminergic dysfunction parallels findings reported for *parkin* (PARK2) patients,^{11,12} another recessive form of early-onset parkinsonism, and suggests that significant dopamine cell loss occurs early in life in patients with recessive parkinsonism. Disease then may progress slowly enough to allow currently uncertain compensatory mechanisms to develop. It is known that in idiopathic PD there is increased dopamine turnover in surviving striatal terminals.¹³ In addition, Whone and colleagues have reported recently increased pallidal dopamine storage in early PD cases.¹⁴ These mechanisms plus altered production of nondopaminergic neurotransmitters may all operate to maintain motor status. Our PARK6 sample size was small, however, and we cannot exclude the fact that the pattern of nigrostriatal dopamine dysfunction that we found in these carriers may be specific to our two families. Our results require confirmation with further larger PARK6 cohorts.

We also found nigrostriatal dysfunction in three members of the two families, who carried a single mutant PARK6 allele and as a group had significant reduction in mean putamen ^{18}F -dopa uptake. Preclinical nigrostriatal dysfunction has been reported previously in asymptomatic cotwins of PD patients¹⁵ and at-risk adult members of unrelated kindreds with familial parkinsonism,¹⁶ one third of whom subsequently developed clinical disease. Our results indicate that reduced presynaptic dopamine terminal function is present in asymptomatic adult PARK6 heterozygotes. Similar observations of reduced striatal ^{18}F -dopa uptake have been reported in heterozygotes carrying a single mutant allele of the *parkin* gene.^{11,12}

A possible molecular explanation for the findings of abnormal nigrostriatal dysfunction in heterozygous PARK2 and PARK6 carriers could be either *haploinsufficiency*¹⁷ such that a single mutant allele results in a reduction of up to 50% of enzymatic activity, which may not be sufficient for normal nigrostriatal activity, or a *dominant negative* effect,¹⁷ in which the nonfunctional mutant polypeptide physically interferes with the function of the normal polypeptide, suggesting that

dimerization or oligomerization of the gene product is requisite for normal function.

Whether or not these PARK6 heterozygotes with subclinical dysfunction will develop clinical parkinsonism over time is unknown. Repeated observation with clinical examination and repeat scanning over time in a much larger cohort will be necessary to confirm these findings.

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Primary Dystonia: Is Abnormal Functional Brain Architecture Linked to Genotype?

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The DYT1 dystonia mutation is associated with an abnormal metabolic brain network characterized by hypermetabolism of the basal ganglia, supplementary motor area, and the cerebellum. In this study, we quantified the activity of this network in carriers of other dystonia mutations to determine whether this functional abnormality is linked to genotype. The findings suggest that the DYT1 metabolic topography is not genotype specific and may be present in carriers of other dystonia mutations.

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Dystonia is a movement disorder characterized by sustained muscle contractions with twisting and repetitive movements or abnormal postures.¹ A common cause of

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