FAMILIAL PARKINSONISM [PARKINSON'S DISEASE AND EARLY ONSET PARKINSONISM]

A genetic, clinical study and ^{18}F-dopa PET study

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In the Faculty of Medicine
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ABSTRACT

This thesis reports a study of familial parkinsonism using diverse scientific tools of both molecular genetics and positron emission tomography functional imaging. Population-based allelic association studies are also described.

Molecular and clinical characterisation of the largest British kindred with autosomal Parkinson's disease (PD).

Genome wide linkage analysis was used to map the sub-chromosomal location of the disorder as the first step for disease-gene identification. The disease mapped to a 50cM region on the short arm of chromosome 12 overlapping a locus, PARK8 that had just been reported in a Japanese kindred, with an identical phenotype. A detailed clinical study of the British kindred identified unilateral onset of tremor in the leg, prominent foot dystonia and behavioural disorder. Intact cognition and sustained levodopa response, was observed despite lengthy disease duration.

Phenotypic study of PARK2 / parkin disease

This case series reports a detailed clinical assessment of twenty four cases of early onset parkinsonism with mutations in PARK2, emphasising the clinical features, atypical phenotypes including cervical dystonia, autonomic dysfunction, peripheral neuropathy, pure exercise-induced dystonia and behavioural disorder prior to the onset of parkinsonism. Olfaction was normal compared to a group of parkin negative patients and idiopathic PD cohorts. A number of unaffected relatives who were parkin heterozygotes had psychiatric symptoms and some had extrapyramidal signs that did not fulfil Queen Square Brain Bank criteria.

^{18}F-dopa Positron Emission Tomography in Familial Parkinsonism.

Functional imaging in cohorts of patients with parkin (PARK2) and PINK1 (PARK6) mutations identified patterns of nigrostriatal dysfunction that was bilateral and

uniform unlike that seen in idiopathic PD. Serial ^{18}F-dopa PET used to assess disease progression in parkin disease over a ten year period, showed that the rate of loss of dopaminergic function was slower compared to idiopathic PD. Asymptomatic parkin and PINK1 heterozygotes also had nigrostriatal dysfunction implying that the gene products exhibited 'haploinsufficiency'.

Population based Allelic Association studies.

A large study using pathologically proven PD cases and controls failed to replicate a report of positive association between an alpha synuclein polymorphism and PD. In a separate study the angiotensin converting enzyme gene was used as a candidate disease gene.

Familial parkinsonism encompasses a heterogeneous group of diseases. Familial 'parkinsonism' was observed in early onset, recessive disease with atypical phenotypes, normal smell and patterns of nigrostriatal dysfunction and rate of progression of functional imaging unlike that seen in idiopathic PD. Functional imaging in asymptomatic heterozygotes suggested that parkin and PINK1 proteins exhibited the phenomenon of haploinsufficiency.

Familial Parkinson's disease, however, with a typical phenotype and pattern of ^{18}F-dopa uptake similar to PD was observed in an autosomal dominant British kindred. This thesis also confirmed locus heterogeneity in autosomal dominant Parkinson's disease and studied a putative susceptibility allele, ACE, in a population-based study.

PUBLICATIONS RELATED TO THE WORK IN THIS THESIS

Full text versions these papers can be found in section 9.4 of the Appendix

Mutations in the gene (LRRK2) encoding dardarin (PARK8) causing familial Parkinson's disease in a British kindred; clinical, pathological, olfactory and functional imaging data.
Khan NL, Jain S, J Holton, N Pavese, L Eunson, M G Sweeney, M Ganguly, W Gilks, J Vaughan, R Katzenschlager, J Gayton, G Lennox, T Revesz, A Singleton, D Nicholl, D Brook, A J Lees, P Piccini, M Davis, N W Wood. Brain 2005 Dec; 128:2786-96.

Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single parkin mutation.
Khan NL, Scherfler C, Graham E, Bhatia KP, Quinn N, Lees A J, Brooks D J, Wood N W, Piccini P. Neurology 2005 11; 65(1):134-6

Olfactory testing differentiates parkin disease from early onset parkinsonism and Parkinson's disease.
Khan NL, Katzenschlager R, Watt Bhatia KP, Wood NW, Quinn N, Lees AJ. Neurology 2004 Apr 13; 62(7): 1224-6.

Parkin disease a phenotypic study of a large case series.
Khan NL, Graham E, Critchley P Schrag AE, Wood NW, Lees AJ, Bhatia KP, Quinn N. Brain. 2003 June; 126: 1279-92.

Clinical and Subclinical Dopaminergic dysfunction in autosomal recessive PARK6-linked parkinsonism: an ^{18}F-dopa PET study.
Khan NL, Valente EM, Bentivoglio AR, Wood NW, Albanese A, Brooks DJ, Piccini P. Annals of Neurology 2002 52; (6): 849-53.

Progression of Nigrostriatal dysfunction in a parkin kindred: an ^{18}F-dopa PET and clinical study.
Khan NL, Brooks DJ, Pavese N, Sweeney MG, Wood NW, Lees AJ, Piccini P.
Brain 2002; 125: 2248-2256.

Parkinson's disease is not associated with the combined alpha-synuclein/apolipoprotein E susceptibility genotype.
Khan N, Graham E, Dixon P, Morris C et al. Annals of Neurology 2001 May; 49(5); 665-8.

Two large British Kindreds with familial Parkinson's disease: a clinicopathological and genetic study.
Nicholls D, Vaughn J, Khan NL, Ho SL, Aldous DE, Lincoln S, Farrer M, Gayton JD, Davis MB, Piccini P, Daniel SE, Lennox GG, Brooks DJ, Williams AC, Wood NW. Brain 2002 January; 125: 1-14.

OTHER PUBLICATIONS THAT AROSE DURING WORK ON THESIS

Parkinsonism and dopaminergic dysfunction is associated with spinocerebellar ataxia type 6 (SCA6).
Khan NL, Giunti P, Sweeney M G, Scherfler C, Brien MO, Piccini P, Wood NW, Lees AJ. Movement Disorders. 2005 Sep; 20(9):1115-9.

Parkin disease in a Brazilian kindred; manifesting heterozygotes and clinical follow-up over ten years.
Khan NL, Horta W, Eunson L, Graham E, Johnson J O, Chang S, Davis M, Singleton A, Wood N W, Lees A J, Movement Disorders. 2005 Apr; 20(4): 479-84

Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease.
Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, Lopez de Munain A, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB. Neuron. 2004 Nov 18;44(4):595-600.

Striatal and cortical pre- and postsynaptic dopaminergic dysfunction in sporadic parkin-linked parkinsonism.
Scherfler C, Khan NL, Pavese N, Eunson L, Graham E, Lees AJ, Quinn NP, Wood NW, Brooks DJ, Piccini P. Brain. 2004 Jun;127(Pt 6):1332-42.

Autosomal Recessive DYT-2-like Primary torsion dystonia: a new family.
Khan NL, Wood N, Bhatia K. Neurology. 2003 Dec 23; 61(12): 1801-3.

Defining the ends of Parkin exon 4 deletions in two different families with Parkinson's disease.
Clarimon J, Johnson J, Dogu O, Horta W, Khan NL, Lees A J, Hardy J, Singleton A. Am J Med Genet B Neuropsychiatric Genetics 2005 Feb 5; 133(1): 120-3.

The tau locus is not significantly associated with pathologically confirmed sporadic Parkinson's disease.
De Silva R, Hardy J, Crook J, Khan NL, Graham EA, Morris CM, Wood NW, Lees AJ. Neuroscience Letters 2002; 330: 201-203.

No pathogenic mutations in the synphilin-1 gene in Parkinson's disease.
Bandopadhyay R, de Silva R, Khan NL, Graham E, Vaughan J, Engelender S, Ross C, Morris H, Morris C, Wood N W, Daniel S, Lees A. Neuroscience Letters 2001 Jul 13;307(2): 125-7.

The Genetics of Parkinson's disease.
De Silva H R, Khan NL, Wood N W. Current Opinion Genetic Development. 2000 Jun; 10(3): 292-8.

Prader-Willi and Angelman syndromes: update on genetic mechanisms and diagnostic complexities.
Khan NL, Wood N W, Current Opinion Neurology 1999 Apr.: 12(2): 149-154

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ABBREVIATIONS

A	adenine
ACE	angiotensin converting enzyme
ADPD	autosomal dominant Parkinson's disease
Ala	alanine
APS	ammonium persulphate
AR-JP	autosomal recessive juvenile parkinsonism
AR	autosomal-recessive
ATP	adenosine triphosphate
bp	base pair
C-	carboxy
C	cysteine
CEPH	Centre d'Études du Polimorphisme Humaine
cM	centimorgan
CR	controlled release
dNTP	deoxynucleotide triphosphate
DAT	dopamine transporter
DJ-1	PARK7 gene
DNA	deoxyribonucleic acid
DRD	dopa-responsive dystonia
DRPLA	dentatorubropallidoluysian atrophy
DYT-1	dystonia gene symbol
DZ	dizygotic
EDTA	ethylene diamine tetra-acetate
EOPD	early onset parkinsonism
^{18}F-dopa	6-[^{18}F] fluoro-L-3-4-dihydroxyphenyalanine
G	glycine
HGMP	Human Genome Mapping Project
http	hypertext transmission protocol
HD	Huntington's disease
H&Y	Hoehn and Yahr
IBR	in between ring
Ile	isoleucine
IPD	idiopathic Parkinson's disease
kb	kilobase
Ki min^{-1}	^{18}F-dopa uptake expressed as an influx constant
LOD	logarithm of odds ratio
LRRK2	leucine rich repeat kinase
Lys	lysine
Mb	megabase
MBq	millibequerels
Met	methionine
mM	millimolar
MMSE	Mini Mental State Examination
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
MRC	Medical Research Council
mRNA	messenger ribonucleic acid
MZ	monozygotic
NCBI	National Centre for Biotechnology Information

NHNN	National Hospital for Neurology and Neurosurgery, London
nm	nanometer
OD	optical density
OMIM	On-line Mendelian Inheritance in Man database
p	short arm chromosome
PARK1-8	Parkinson's disease gene symbols 1-8
PARK2	parkin disease gene
PINK1	PTEN (phosphatase and tensin homolog deleted on chromosome ten) induced kinase-1
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDI	peripheral dopa decarboxylase inhibitor
PET	positron emission tomography
PD	Parkinson's disease
Pro	proline
PSP	progressive supranuclear palsy
q	long arm chromosome
R	arginine
RING	really interesting new gene
ROI	region of interest
rpm	revolutions per minute
SCA3	spinocerebellar ataxia type 3
SD	standard deviation
SDS	sodium sulfate dodecyl
SNCA	alpha synuclein gene
SNP	single nucleotide polymorphism
SPECT	single-photon emission computerised tomography
T	thymine
TBE	tris-borate EDTA solution
TE	tris EDTA solution
TEMED	tetramethylthylenediamine
Thr	treonine
UCHL1	ubiquitin carboxy terminal hydrolase 1
UPDRS	unified Parkinson's disease rating scale
UPSIT	University of Pennsylvania smell identification test
VNTR	variable number tandem repeats
UV	ultraviolet
Y	tyrosine
Z	LOD score

1. INTRODUCTION

Over the last decade the identification of disease-causing genes in familial parkinsonism (autosomal recessive early-onset parkinsonism and autosomal dominant Parkinson's disease) represents an astounding achievement by the scientific community. Until the latter part of the twentieth century, Parkinson's disease was thought not to have a genetic aetiology and was attributed to acquired factors such rural living, pesticide consumption and the drinking of well water (Hubble et al., 1993). To date over ten loci and seven genes have been identified in familial parkinsonism. As a result, functional studies of the aberrant protein encoded has for the first time, contributed to a significant understanding of the molecular mechanism underlying neurodegeneration in Parkinson's disease.

Idiopathic PD is one of the most common neurodegenerative disorders, with a prevalence of 1 to 2 percent after the age of 65 years (De Rijk et al, 1997). The phenotype is characterised by resting tremor, rigidity, bradykinesia with olfactory dysfunction being as common as the pill-rolling tremor (Katzenschlager et al., 2004). Levodopa responsiveness is characteristic. A concrete diagnosis is posthumous; the pathognomonic feature being eosinophilic cytoplasmic inclusions Lewy bodies, which distinguishes Parkinson's disease from other forms of parkinsonism. Whilst a definitive diagnosis is posthumous, the use of functional imaging using PET tracer ^{18}F-dopa has been instrumental in establishing the diagnosis in patients by identifying characteristic patterns of disruption of presynaptic dopaminergic function. In Parkinson's disease there is relative sparing of the function of the head of caudate and anterior putamen compared to severe involvement of the posterior putamen (Brooks et al., 1990). Idiopathic, 'later onset' PD represents a 'complex trait' that is mostly non-

Mendelian and multi-factorial in aetiology with the interaction of one or more susceptibility genes together with 'environmental' influences.

Politician turned physician, James Parkinson, was the first to publish a clinical description of Parkinson's disease in 'An Essay on the Shaking Palsy' in 1817:

'tremulous motion with lessened voluntary muscular power in parts, not in action'

'a propensity to bend the trunk forwards and to pass from walking to a running pace'

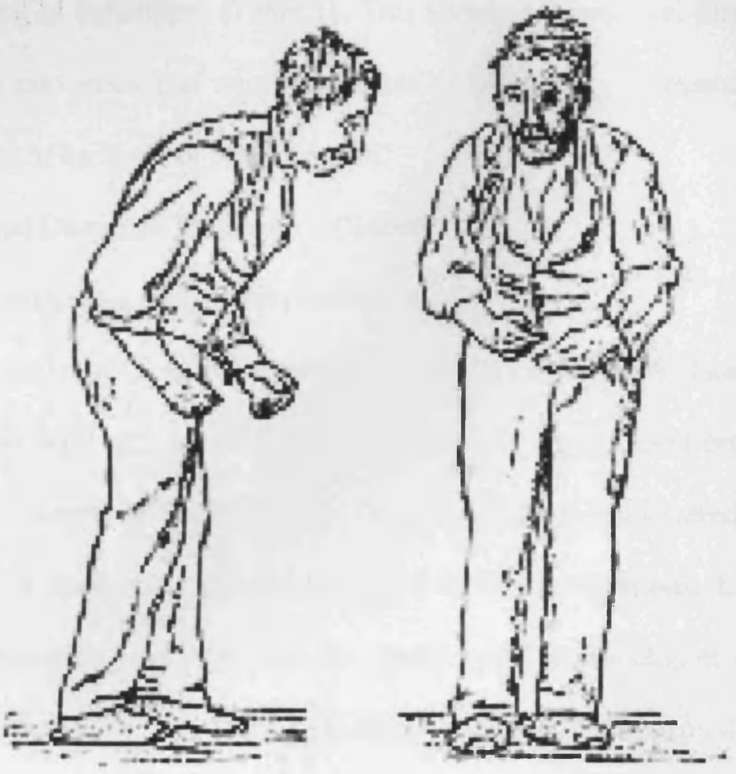
Forty years later, Jean Martin Charcot added rigidity to the clinical description and called it 'Parkinson's disease'.

1.1 Mendelian Parkinson's disease and parkinsonism.

Evidence of a hereditary contribution to the disease dates as far back as 1880, when Gowers (Figure 1.1a and b) observed that patients with Parkinson's disease often had an affected relative (up to 15% of his patients) and based on this he concluded that the disease was attributable to 'hereditary factors' (Gowers et al., 1886). A century later, different scientific tools (epidiemiological studies, twin studies, functional imaging) have continued to propogate this theory; recent community-based case-control studies estimate the frequency of Parkinson's disease among relatives of cases to be between 6 and 33% (Lazzarini et al, 1994; Payami et al, 1994; De Michele et al, 1996; Marder et al, 1996; Payami et al, 2002). Whilst the most recent twin study found similar, overall concordance rates for PD in a total of 19,842 white male twins, a subset analysis of twin pairs (monozygotic versus dizygotic) with diagnosis at or before the age of 50 years showed a relative risk of 6 (95% confidence interval 1.69-21.26) (Tanner et al, 1999). ^{18}F-dopa PET has been used in monozygotic and dizygotic twin pairs who were, at baseline, clinically discordant for PD.

Figure 1.1a Sir Richard William Gowers (1845-1915) both Artist and Physician at University College, London and the National Hospital for Neurology
(Courtesy of audio-visual department, National Hospital for Neurology and Neurosurgery, London)

Figure 1.1b
An illustration drawn by Gowers in 1886 as part of his documentation of Parkinson's disease. The image is taken from his book *A Manuel of Diseases of the Nervous System* (London, J & A Churchill 1886-1888).



Subclinical dopaminergic dysfunction was found to be significantly higher in MZ twins compared to DZ twins, moreover the MZ twin was more likely to develop PD, suggesting a substantial role for inheritance in 'idiopathic' PD (Piccini et al, 1999).

The year 1996, represents a landmark year in which the first locus was identified in familial PD to the present day, there has been an explosion in the number of disease-genes or linked loci implicated in recessive forms of parkinsonism (*PARK2/parkin, DJ1, PINK1*) and dominant forms of Parkinson's disease (*SNCA, UCHL1, LRRK2* and the chromosme 2p haplotype) (Table 1). This section outlines loci identified to date dividing them into autosomal dominant forms of Parkinson's disease and autosomal recessive forms of early-onset parkinsonism.

1.1.1 Autosomal Dominant Parkinson's Disease

PARK1; point mutations and multiplications in SNCA

The first susceptibility locus for autosomal dominant Parkinson's disease was linked to chromosome 4q21-q22 in 1997, in an Italian-American kindred originating from Contursi, near Salerno in Southern Italy. This is the largest PD kindred characterised to date, with at least sixty affected members in four generations. Linkage of the disease to chromosome 4q21-q23 in this family (Polymeropoulos et al, 1996) was followed by identification of a G>A transition at position 209 in exon 4 of the SNCA gene, causing an alanine to threonine substitution (Ala53Thr) in the α-synuclein protein (Polymeropoulos et al, 1997).

Designated "Parkinson's disease type 1" (PARK1), the phenotype is similar to PD with Lewy bodies at post-mortem and a typical pattern of nigrostriatal dysfunction using^{18}F-dopa PET (Samii et al, 1999). Atypical features however included a

Table 1. The Mendelian forms of Parkinson's disease and parkinsonism

Locus	**Location**	**Gene**	**Inheritance**	**Details**
PARK1	4q21	*α-synuclein*	AD	i) point mutations: Italian-Greek A53T / German A30P / Spanish E46K ii) multiplications: 4 copies early-onset, PD and Dementia with Lewy bodies, variable pathology: PD with LBs; DLBD; glial α-syn inclusions
PARK2	6q25-27	*parkin*	AR	early-onset, slowly progressive, L-dopa sensitive, hyperreflexia, dystonia, early and late onset parkinsonism, manifesting heterozygotes, worldwide
PARK3	2p13	x	AD	German family only typical idiopathic PD with pathology with LBs
PARK4	4q21	*α-synuclein*	AD	incorrectly linked; found to be an α-synuclein triplication now PARK1
PARK5	4p14	*UCH-L1*	AD	German I93M single family only
PARK6	1p35-36	*PINK1*	AR	early onset and onset typical of idiopathic PD, no pathology, rare
PARK7	1p36	*DJ-1*	AR	early onset, slow progression, dystonia, similar to parkin, rare
PARK8	12p11	*LRRK2*	AD	phenotype identical to idiopathic PD, remarkable variation in pathology
PARK9	withdrawn / not just parkinsonism			'Kufor-Rakeb syndrome' one consanguineous Jordanian family with AR parkinsonism, spasticity, dementia & supranuclear palsy
PARK10	1p32	x		susceptibility locus identified by genome wide linkage study of 51 Icelandic families with 1 or more family members idiopathic PD
PARK11	2q	x		susceptibility locus identified by genome linkage in multiplex US families

X = gene unknown AD = autosomal dominant, AR = autosomal recessive relatively early age of onset of illness, at a mean of 46 years (±13.0), a relatively rapid progression of clinical disease, myoclonus, dementia and hypoventilation (Golbe et al., 1990)

Other familial and isolated cases of PD with an identical base pair substitution in SNCA have been identified. Almost all have ancestry that can been traced back to Salerno, Italy or to the Peloponnese region in Greece (two areas in the Mediterranean geographically linked by a historical trade route) suggesting that this mutation arose from a single founder and is therefore a rare cause of familial Parkinson's disease. (Polymeropoulos et al., 1997; Athanassiadou et al., 1999; Papadimitriou et al., 1999). Two additional novel point mutations have been discovered in unrelated German and Spanish families (Kruger et al., 1998, Zarranz et al., 2004). The phenotype in the Spanish family had striking clinical features consistent with diffuse Lewy body disease (visual hallucinations, dementia) with a large number of cortical and sub-cortical Lewy bodies (Zarranz et al., 2004).

The PARK1 locus was examined by a number of different groups: no mutations were identified in a large number of sibling pairs and autosomal dominant families with PD (Bennett et al., 1998; Farrer et al, 1998; Vaughan et al, 1998a and b) confirming that point mutations in *SNCA* are a rare cause of PD.

Mutant *α–synuclein* transcript exhibits 'toxic gain of function'; an abnormal intracellular accumulation of aberrant protein that inhibits proteosomal function resulting in Lewy body formation and a selective vulnerability of nigral dopaminergic neurones (Erikson et al., 2003).

Multiplications of *SNCA*, overproducing wild type protein rather than a mutant product have also been shown to cause familial PD. Triplications of *SNCA* (resulting in four normal copies of the gene rather than two) also causes autosomal dominant

Parkinson's disease (Singleton et al., 2003) and was first reported in the North American 'Iowan kindred' (previously designated as *PARK4)* whose phenotype was characteristic of dementia with Lewy bodies than rather than Parkinson's disease with a mean age of onset of 33 years and several atypical features such as early weight loss, dysautonomia and dementia (Muenter et al., 1998). Cardiac denervation has also been reported in this kindred (Singleton et al., 2004). Neuropathological changes included nigral degeneration and Lewy-body formation, but additional conspicuous vacuoles in the hippocampus.

Duplications have also been detected in other unrelated PD kindreds (Ibanez et al., 2004, Chartier-Harlin et al., 2004)) however multiplications are rare causes of PD (Johnson et al., 2004). Pathogenicity has been confirmed in animal models that have shown over-expression of wild-type alpha synuclein is directly toxic to dopaminergic neurones (Masliah et al., 2000, Feany et al., 2000).

Despite the rarity of SNCA mutations in familial PD, the protein alphasynuclein, has become the centrepiece of the understanding of the Lewy body related diseases; alpha-synuclein protein is a major fibrillar component of Lewy bodies and Lewy neurites found in PD, Lewy Body Dementia and some cases of Alzheimer's disease (Spillantini et al, 1997) and the glial cell inclusion bodies of Multiple System Atrophy (Tu et al, 1998). These disorders have therefore been called "synucleinopathies" (Duda et al, 2000).

Lewy bodies are spherical structures with radially arrayed intraneuronal aggregations of antigenic components, including various proteins, fatty acids, sphingomyelin and polysaccharides. Alpha-synuclein and ubiquitin appear to be the major constituents of Lewy bodies, although the latter is not always present (Spillantini et al, 1997).

Immunostaining for alphasynuclein has now become the most sensitive means of demonstrating Lewy bodies.

These rare families with mutations in SNCA, prove that both abnormal alpha-synuclein and the overproduction of normal protein can directly cause Parkinson's disease. It is still unclear however if alpha-synuclein has a direct causative role in the more common forms of Parkinson's disease, or whether it simply represents an end stage marker of the underlying pathogenic process.

PARK3 linkage to chromosome 2p13

Six families of European origin with autosomal dominant PD have been linked to chromosome 2p13. The phenotype is not dissimilar to idiopathic PD, with age of onset ranging from 37 to 89 years however dementia occurred in two of the kindreds (Gasser et al, 1998). Postmortem analysis from three of the families showed degeneration of dopaminergic neurones in the substantia nigra with Lewy bodies but there was also Alzheimer-like pathology. The disease gene at this locus has yet to be identified.

PARK5 mutations in UCH-L1

There are several reasons why genes encoding ubiquitin would be strong candidate genes in Parkinson's disease. Lewy bodies contain many multi-ubiquitinated chains arising from incomplete degradation of proteins in the proteasomal pathway. Ubiquitin C-terminal hydrolase L1 (UCH-L1) is a member of a gene family whose products hydrolyse small C-terminal of ubiquitin to generate the ubiquitin monomer. Expression of UCH-L1 (which represents 1 to 2% of total soluble brain protein) is highly specific to all neurons and to cells of the diffuse neuroendocrine system and their tumours (Solano et al, 2000), and is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative disease (Lowe et al, 1990). An

Ile93Met missense mutation in the UCH-L1 gene was identified in a German family with familial PD; two German brothers with L-dopa responsive Parkinson's disease and family history compatible with autosomal dominant but incompletely penetrant Parkinson's disease (Leroy et al., 1998).

Functional studies of Ile93Met UCH-L1 and wild-type enzymes showed that the mutant had nearly a 50% reduction in activity (Leroy et al, 1998). However, a mutation screen of a large cohort of PD families failed to identify any mutations within the UCH-L1 gene (Harhangi et al, 1999; Lincoln et al, 1999b; Wintermeyer et al, 2000). The role of this gene in the pathogenesis of autosomal dominant PD remains undefined.

PARK8 mutations in LRRK2

A third locus for an autosomal dominantly inherited phenotype resembling late-onset Parkinson's disease was first mapped to chromosome 12 (PARK8) in a large Japanese family; nigral degeneration without Lewy bodies was reported in this family (Funayama et al., 2002) Subsequently, further evidence for independent mutations at this locus provided by the report of a linkage study in, Family D from North America, (Zimprich et al., 2004). PARK8 is the subject of chapters 2 and 3 where it will be discussed in greater detail.

1.1.2 Other loci in Familial Parkinson's disease: NURR1 and Synphilin-1

NR4A2 (NURR1) mutations have been identified in ten patients with late-onset autosomal dominant Parkinson's disease (Le et al., 2003). However, NR4A2 knockout mice do not develop nigral dopamine neurones but heterozygous knockouts appear to have an increased sensitivity to MPTP neurotoxicity (Zetterstrom et al., 1997). Follow-up reports have failed to find additional pathogenic NR4A2 mutations

(Hering et al., 2004, Nichols et al., 2004) questioning the significance of non-coding variants identified in the NR4A2 gene.

Synphilin-1

Synphilin-1 is a component of the Lewy body, which interacts with both α– synuclein and parkin, and may have a functional role in the ubiquitin mediated degradation pathway (Engelender et al., 1999). A missense mutation of the gene has been reported in two German patients with sporadic Parkinson's disease that share the same rare haplotype, indicating common ancestry however significance of this finding is also unclear (Marx et al., 2003).

1.1.3 Autosomal Recessive Parkinsonism

Mutations in PARK2 / Parkin

Parkin is the product of a gene, *PARK2*, which spans more than 500 kb with 12 coding exons and an open reading frame of 1,395-bp. The protein has 465 amino acids with moderate homology to ubiquitin at the amino terminus and two RING-finger motifs at the carboxy-terminus (Kitada et al, 1998) and flanking an IBR (In Between Ring) region. These domains are typical of proteins which act as ubiquitin-ligase. Functional *in vitro* studies have demonstrated that parkin acts as an ubiquitin-ligase (E3), and is requisite in the ubiquitin-proteasome degradation pathway (Shimura et al, 2000). This pathway contributes to cell survival by degrading abnormal misfolded proteins that would otherwise precipitate within the endoplasmic reticulum and lead to cell toxicity and cell death. In order to be degraded by the proteasome, abnormal proteins are tagged with a polyubiquitin chain and incorporated within the 26S proteasome and degraded. Free polyubiquitin chains are then degraded by ubiquitin-hydrolase enzymes (such as UCH-L1) and ubiquitin molecules can be recycled.

In brains of control individuals, the parkin protein is widely expressed, mainly in the pigmented neurons of the substantia nigra and locus coeruleus. Histopathology of patients with idiopathic, 'late onset' PD has demonstrated that parkin is a major component of the Lewy bodies in addition to apha-synuclein, ubiquitin and other proteins.

Autosomal recessive early onset parkinsonism (EOPD), first described in 1973 in Japan, where it is also called autosomal recessive juvenile parkinsonism (ARJP), is often characterised by dystonia at onset, hyperreflexia, early complications from L-Dopa treatment (in contrast to dopa responsive dystonia-DRD) and slow progression (Yamamura et al., 1973). PARK2 was the first recessive parkinsonism locus to be mapped and cloned (Kitada et al., 1998). Deletions, multiplications, or point mutations in the coding regions have been identified not only in kindreds of different ethnic origins, but also in isolated cases of young onset parkinsonism and familial cases with an age of onset as late as 64 years (Abbas et al., 1999, Lucking et al., 2000, Klein et al., 2000).

Neuropathological examination has been reported (Mori et al., 1998, Hayashi et al., 2000, van de Warrenburg et al., 2001, Farrer et al., 2001). Neuropathology usually does not have Lewy bodies although two reports describe Lewy body inclusions (Chen et al, 2000; Farrer et al, 2001).

In remaining cases there was an absence of Lewy bodies and a severe and generalised loss of dopaminergic neurons from the substantia nigra pars compacta and locus coeruleus. One of these showed additional neurofibrillary tangles and argyrophilic astrocytes in cerebral cortex and brainstem nuclei (Mori et al, 1998), the second showed additional involvement of the substantia nigra pars reticulata (Hayashi et al, 2000), and the third showed neuronal loss in parts of the spinocerebellar system (van de Warrenburg et al, 2001). Thus, 'parkin disease' is a distinct genetic entity whose clinical and pathological features show varying degrees of overlap with those of idiopathic Parkinson's disease (IPD).

"Parkin disease" may be a separate pathological entity with clinical features that overlap with Parkinson's disease. It is presumed that PARK2 mutations are 'loss-of-function'; unlike controls cases, the protein is absent in brains of patients with parkin disease (Hayashi et al, 2000) suggesting that there is a breakdown in the ubiquitin-proteasome machinery; the consequent accumulation of proteins that cannot be ubiquitinated and degraded leads to a cascade of endoplasmic reticulum stress and selective neuronal degeneration. This may well explain the absence of Lewy bodies, which are mainly composed of ubiquitinated aggregates (Nussbaum, 1998; Giasson and Lee, 2001).

PARK2 is by far numerically the most important of the recessive loci contributing to early onset parkinsonism; parkin mutations account for 45% of autosomal recessive parkinsonism with an age of onset <45 years and 77% of isolated cases of parkinsonism with an age of onset <20 years (Lucking et al., 2000) but remains a rare cause of parkinsonism in patients with onset over 40years of age (Oliveira et al., 2003).

Mutations in PINK1 (PARK6)

Mutations in the PTEN (phosphatase and tensin homolog deleted on chromosome ten) induced kinase-1 gene *(PINK1)* also causes early onset parkinsonism (Valente et al., 2004). The *PINK1* gene encodes a putative protein-kinase. These enzymes phosphorylate target proteins, which then perform important cellular roles such as signal transduction.

Wild type and mutant PINK1 protein primarily locates to the mitochondrion (Valente et al., 2004) and protects cells from stress-induced mitochondrial dysfunction and apoptosis.

The phenotype of *PINK1* associated parkinsonism has greater overlap with the clinical features of idiopathic Parkinson's disease compared to parkin or *DJ-1* associated disease (Valente et al., 2004, Valente et al., 2005)

Mutations in DJ-1 (PARK7)

This locus was first linked to parkinsonism on the short arm of chromosome 1 (25 cM telomeric to PARK6) in a large consanguineous family, originating from an isolated community living in the Netherlands (van Duijn et al, 2001). A homozygous deletion in gene, *DJ-1,* was subsequently identified in this Dutch kindred and a homozygous missense mutation resulting in the substitution of a highly conserved leucine at position 166 by a proline, in affected subjects in a consanguineous Italian family (Bonifati et al, 2003).

DJ-1 contains 8 exons, of which the first two are non-coding and alternatively spliced in mRNA. One major transcript encodes a protein that is ubiquitously expressed in body tissues and brain. Wild-type DJ-1, under oxidative stress locates to the outer mitochondrial membrane maintaining neuronal integrity and survival (Canet-Ariles et al., 2004). The DJ-1 phenotype includes an early age of onset with individuals from the original Dutch kindred reporting an age of onset from 27 to 40 years. Disease progression was slow with some patients refusing treatment, owing to mild disease. Those in whom treatment was instigated with levodopa or dopamine agonists, showed a good response in addition to off-dystonia, levodopa-induced dyskinesias and fluctuations. Behavioural disorder (psychosis and neurosis) was also noted as was short stature and brachydactyly (Dekker et al., 2004). Functional imaging has shown a pattern of nigrostriatal dysfunction that is unlike idiopathic PD (Dekker et al., 2004a). Pathology in this locus has yet to be reported. Mutations in DJ-1, causing early-onset disease, have a prevalence of approximately one percent (Abou-Sleiman et al., 2003).

1.2 Scientific tools used in this thesis.

This section outlines in detail two very different scientific tools used in this thesis; molecular genetics and functional imaging using ^{18}F-dopa positron emission tomography. At the start of this thesis, this somewhat unusual combination was initially used as a novel method to increase the power of linkage analysis by detecting preclinical disease in asymptomatic relatives in a British kindred with autosomal dominant Parkinson's disease. This would extend the phenotype and increase the number of 'affected subjects' in a genome-wide search and in linkage analysis. At the beginning of my thesis, in 1998 the use of functional imaging to study both disease and presymptomatic subjects in mendelian parkinsonism was novel and was therefore employed in other projects.

1.2.1 Identifying disease-genes

There is no standard procedure for gene identification but whichever route is adopted the key step is to arrive at a plausible 'candidate gene' which can then be tested for mutations in diseased individuals (Strachan 2000). In principle methods be divided into position independent strategies that do not require chromosomal location or positional cloning methods that rely on information of the chromosomal location.

Position-independent approaches include methods starting with the protein product (using amino acid sequences used to generate specific oligonucleotide probes), functional cloning (expressing random fragments of DNA and isolating fragments that cause a desired change) or selecting a candidate gene based on its potential contribution to the pathophysiology of the disease and screening either populations (unrelated patients, sibling pairs, twins) using allelic association studies or individual patients using sequencing methods.

Position-dependent approaches refer to 'positional cloning' in which the initial step in identifying the disease-causing gene, is to map its chromosomal location first (using genome-wide studies in either populations, sibling pairs or dominant or recessive kindreds). This enables the refined subchromosomal location to be screened for candidate genes in which a disease-causing mutation is identified in affected subjects. In reality parallel approaches are used and both converge on mutation testing in a candidate gene.

The two methods used in this thesis were linkage analysis in a dominant kindred with PD and population based association studies.

At a fundamental level genetic association and linkage analysis rely on similar principles; both rely on the co-inheritance of adjacent DNA variants. Linkage relies on identifying haplotypes inherited over several generations in families of more recently observed ancestry where fewer recombinations have occurred such that disease gene regions are large encompassing many megabases. Association relies on the retention of adjacent DNA variants over many generations from historic recombinations such that disease-gene regions are theoretically small (Lon et al., 2000). In a sense, association studies can be regarded as very large linkage studies.

1.2.1.1 Population based allelic association studies.

Association studies test whether a genetic marker (polymorphism or allele) occurs more frequently in unrelated, diseased cases than in age and sex matched controls. If a truly significant association emerges (disproportional over-representation amongst diseased cases) the polymorphism itself is the susceptibility locus, or in linkage disequilibrium with the susceptibility locus. In either case it should enable disease mapping and the identification of people at risk of developing disease.

Linkage disequilibrium (also known as gametic phase disequilibrium or allelic association), refers to the non-random association of alleles such that particular DNA variants (including a disease mutation) remain together on ancestral haplotypes for many generations. The power (the probability of correctly detecting a genuine association) of population-based studies can be much greater than linkage.

If a positive association emerges it requires confirmation in an independent cohort, preferably with larger study numbers with stringent standardisation of controls samples (Bird et al., 2001). Failure of replication does not necessarily render the original result false; there are several possible explanations for the discrepancy between two comparable groups including population stratification (populations containing several genetically distinct subsets), statistical artefact including subgroup analysis and multiple testing, small sample sizes that exacerbate locus heterogeneity and are prone to random error, incorrect phenotypes, poorly matched controls (using retrospective cohorts prone to selection bias rather then prospective cohorts) and the biological credibility of the gene-allele-phenomenon-disease association (Strachan and Read., 1999, Gambaro et al., 2000).

Association studies in PD to date have broadly focussed on two types of candidate genes. Firstly those primarily involved in oxidative stress, xenobiotic toxicity, altered dopamine metabolism or impaired proteasomal degradation in PD. Secondly functional polymorphisms selected from known mendelian genes identified in rare families with autosomal recessive or dominant kindreds. Linkage disequilibrium in populations of 'sporadic' PD or affected sibling pairs has identified several allelic associations although these have not always been replicated in independent cohorts (Tan et al., 2000).

1.2.1.2 Linkage analysis

Linkage analysis makes use of the exception to Mendel's Law of Independent Assortment which states that alleles at different genetic loci assort at random during meiosis; homologous chromosomes cross over and exchange genetic material during recombination such that that 50% of chromosomes will be recombinant, and 50% non-recombinant for these loci. Loci in close physical proximity on the same chromosome, however, tend to be inherited together and are said to be *linked* and alleles on the same small chromosomal segment that tend to be transmitted as a block thorough a pedigree is known as a haplotype. Haplotypes mark chromosomal segments which can be tracked through pedigrees and through populations. Hence during a genome wide search, when DNA markers (with a known location) on the human genetic map, co-segregates with the disease (only affected subjects in a pedigree), linkage exists, and the DNA marker lies in close proximity to the disease gene.

Genetic mapping using linkage analysis has essential requirements including monogenic mode of inheritance that can be established by segregation analysis, correct phenotypic designation of affected and unaffected status.

The LOD score method (Morton, 1955), a maximum likelihood analysis, calculates the probability that two loci are linked, expressed as a LOD score which is a $\log_{10}$ of the odds ratio favouring linkage. Convention dictates that a LOD score > 3, which indicates a probability in favour of linkage of 1000 to 1, is enough to establish linkage, and conversely a LOD score of -2 indicating a probability against linkage of

100 to 1 excludes linkage between the two loci being tested. Standard LOD score (parametric) analysis requires a precise genetic model, detailing the mode of inheritance, gene frequencies and penetrance of each genotype. The LOD score is calculated for various values of θ using computer programs such as MLINK (Lathrop et al., 1984 and 1988) to obtain the value of θ associated with the highest LOD score. This provides an estimate of the genetic distance between the two loci studied.

Genetic studies of ‘complex traits’ such as PD face difficulties arising from uncertainties in diagnosis, disease definition and lack of understanding of genetic transmission. In addition in Mendelian disease, especially with autosomal dominant inheritance, linkage analysis can be impaired by incomplete penetrance, variable phenotypic expression, genetic heterogeneity, and phenocopies.

In PD, positional cloning methods using linkage analysis in Mendelian kindreds have been the most successful tool in disease mapping of genes identified to date (Table 1).

1.2.2 Functional Imaging and the use of ^{18}Fluoro-dopa Positron Emission Tomography

The cell bodies of dopaminergic neurons in the human mesencephalon are located in the 'nigral complex'; axons from these cell groups, in particular the substantia nigra and ventral tegmental area project to the striatum (caudate and putamen), pallidum as well as the frontal, cingulate and olfactory cortices (Agid et al., 1991). These neurons synthesize dopamine *in situ* from tyrosine which is hydroxylated by tyrosine hydroxylase to 3,4,-dihydroxy-L-phenylalanine (L-dopa). This enzyme is the rate-limiting step in the synthesis of dopamine; L-dopa is then decarboxylated by aromatic L-amino acid decarboxylase to form dopamine and concentrated within neuronal vesicles by an ATP dependent process. Thereafter, enzymes catechol-*O*-methyl transferase (COMT) and monoamine oxidase (MAO) covert dopamine to homovanillic acid and other metabolites.

In 1983, Garnett and co-workers first reported the use of radiotracer 6-[^{18}F] fluoro-L-3-4-dihydroxyphenyalanine (^{18}F-dopa) in Positron Emission Tomography (PET) to visualise the nigrostriatal dopaminergic system *in vivo.*^{18}F-dopa PET provides a measure of the structural and biochemical integrity of the presynaptic nigrostriatal projection; uptake rate constant is determined by the transfer of dopa across the blood brain barrier, its decarboxylation to fluorodopamine by l-aromatic acid decarboxylase and its retention in nerve terminals (Booij et al., 1999). Although it is not a direct measure of endogenous dopamine synthesis it correlates highly with dopamine levels in the parkinsonian striatum of humans (Pate et al., 1993). However ^{18}F-dopa cannot be used to provide an index of striatal dopamine levels; there is no evidence that ^{18}F-dopa uptake and dopamine levels correlate directly.

After intravenous injection, ^{18}F-dopa tracer in transported across the blood brain barrier and converted to ^{18}F-dopamine by amino acid decarboxylase; ^{18}F-dopamine accumulates in the nigrostriatal projections and is metabolised by monoamine oxidase B and catechol-O-methyltransferase (Piccini et al., 1995). Striatal ^{18}F-dopa uptake is measured as an influx constant, K_i, which reflects the capacity of caudate and putamen to metabolize exogenous ^{18}F-dopa into dopamine thus giving measure of dopamine terminal function (Firnau et al., 1987).

The pattern of ^{18}F-dopa uptake in Parkinson's disease is characterised by a more pronounced reduction of striatal uptake in the putamen than in the caudate nucleus (Leenders et al., 1986, Leenders et al., 1990) which has been confirmed in post-mortem studies (Kish et al., 1988). In addition uptake of radiotracer is asymmetrical and correlates with disease severity in patients with PD; levels of striatal and nigral

^{18}F-dopa uptake at different stages of PD all correlate with clinical disease severity as measured by the unified PD rating scale (UPDRS). ^{18}F-dopa PET has been used as an objective *in vivo* measure of disease progression; Morrish and co-workers found that putamen K_i was the most sensitive measure of disease progression; a mean putamen ^{18}F-dopa uptake 75% of normal was associated with symptom onset with a mean preclinical period up to a decade (Morrish et al., 1998). ^{18}F-dopa PET has also been used in the detection of preclinical disease in PD.

In 1991 Langston and colleagues suggested that Parkinson's disease existed in three different phases. The first was a 'disease free' state in which genetic and environmental risk factors were present. The second was a 'pre-symptomatic phase' in which the disease process had commenced but without clinical manifestation and the third stage was the 'symptomatic phase' (Koller et al., 1991, Langston et al., 1991). It was hypothesized that differences in gene penetrance and expressivity as well as exposure to undefined environmental factors accounted for whether or not progression to a symptomatic, clinical state would occur.

Evidence for a presymptomatic phase has come from both postmortem examination and ^{18}F-dopa PET studies. Histopathology of subjects, who did not have Parkinson's disease, has shown significant neuronal loss and Lewy bodies that are typical of PD (Gibb et al., 1988). In addition, the age-specific prevalence of Lewy bodies increased from 3.8% to 12.8% between the sixth and ninth decade (Gibb et al., 1988); the prevalence of clinical disease is up to 2% in this age group.

^{18}F-dopa PET studies in cynomolgus monkeys injected with MPTP showed significantly reduced ^{18}Fdopa uptake compared to control values (Guttman et al., 1988). These animals subsequently became parkinsonian after repeated injections with MPTP. PET studies in asymptomatic patients exposed to MPTP (Calne et al.,

1985) and those at risk of developing parkinsonism-dementia complex on Guam (Snow et al., 1990) have also shown subclinical nigrostriatal dysfunction. In 1997, Piccini and co-workers published a study that detected significant nigrostriatal dysfuncton, using ^{18}F-dopa PET, in up to 25% of unaffected members from kindreds with familial Parkinsonism and PD; 40% went onto develop frank disease within two years of the initial PET scan (Piccini et al., 1997). Furthermore, a longitudinal ^{18}F-dopa PET study in eighteen MZ and sixteen DZ twins who were clinically discordant for PD showed that subclinical nigrostriatal dysfunction was higher in the MZ twins compared to DZ twins and that all, asymptomatic MZ co-twins showed a loss of dopaminergic dysfunction; 25% developed PD whilst none of the DZ twin pairs became clinically concordant.

THE AIM OF THIS THESIS

This thesis was undertaken during an exciting time in inherited movement disorders when disease-genes were being identified not only familial parkinsonism, but also in dystonia, ataxia and chorea amongst others.

At the start of my research in September 1998, two disease genes had been identified in PD (alpha synuclein and PARK2 'parkin') and by the end, in January 2002 there were ten designated 'PARK' loci. Over this period of time there was an enormous production of molecular genetic data in Mendelian parkinsonism however phenotypes of each locus were less defined, pathology was limited and functional imaging in patients with familial parkinsonism and asymptomatic heterozygotes, was novel.

The aim of this thesis was:

i) to map a novel locus in the largest British kindred with autosomal dominant Parkinson's disease

ii) to assess in detail the phenotype of the British kindred with autosomal dominant PD and compare to other dominant kindreds

iii) to perform a detailed clinical study of 'parkin' patients and asymptomatic parkin heterozygotes

iv) to use ^{18}F-dopa PET to characterise the pattern of nigrostriatal dysfunction in patients and asymptomatic heterozygotes with 'parkin' (PARK2) and PINK1 (PARK6) mutations

v) to identify a susceptibility allele in population-based, allelic association studies

2. CLINICAL FEATURES OF THE LARGEST BRITISH KINDRED WITH AUTOSOMAL DOMINANT PARKINSON'S DISEASE.

2.1 Introduction

The largest British kindred with autosomal dominant Parkinson's disease was identified by Dr Graham Lennox, Consultant Neurologist, Queens Medical Centre, Nottingham who referred this kindred to Professor Nicholas Wood, Institute of Neurology, in 1995. Initial investigations of this kindred traced the ancestry back to the county of Lincolnshire, England; fifteen affected members (six living and nine deceased) were identified (Nichol et al., 2002). A preliminary clinical description of the phenotype was not dissimilar to idiopathic Parkinson's disease with asymmetrical rest tremor and L-dopa responsiveness; pathology was not available at that time (Nichol et al., 2002).

The next chapter describes the molecular characterisation of this kindred, linking the disease to the PARK8 locus on chromosome 12. Subsequent work by my colleagues was successful in cloning the gene at the PARK8 locus and identifying a disease-causing mutation in a novel sequence encoding leucine-rich repeat kinase2, *LRRK2* (Paissan-Ruiz et al., 2004).

The aim of this study were i) to extend the pedigree to identify new affected subjects that would contribute to linkage analysis ii) report follow-up data including a more detailed account of the phenotype and progression of disease.

2.2 Methods

2.2.1 Genealogy and Independent Advertising.

Genealogical data was collected through civil and church records of birth, marriages and deaths, medical records and accounts from family members. This was performed in collaboration with Juliet Gayton, Genealogist, Exeter University, UK.

In addition a variety of methods (coverage by regional news on national television, articles in national newspapers, interviews on regional and national radio and articles in the PDS newsletter) were used to assemble a cohort of familial cases of PD whereby patients or relatives of patients with a family history of PD would volunteer information about their family history (examples shown in Appendix section 8.2).

2.2.2 Patients

All subjects gave informed consent and the study was approved by the Joint Research and Ethics Committees of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, Queen Square, London. A detailed clinical study of these subjects was performed incorporating a standardised clinical proforma. Cognitive assessment incorporated the use of the Folstein Mini-Mental State Examination (Folstein et al., 1975). The University of Pennsylvania Smell Identification Test (UPSIT) was used to test olfaction (Doty et al., 1984). Scores range from 0-40; higher scores denote better olfaction.

2.3 Results

2.3.1 Genealogical data and other families

An extensive genealogical search was performed to identify further affected members in the Lincolnshire kindred. This was successful in identifying another branch in which four members were affected: all were deceased. This did however provide further evidence that the pattern of inheritance was autosomal dominant rather than the chance co-occurrence of PD cases in these families.

800 families with familial Parkinson's disease (two or more first or second degree relatives) were ascertained following advertising methods. To date, none of these families have been genealogically linked to the Lincolnshire kindred by Juliet Gayton Genealogist, Exeter University, UK

2.3.2 Clinical data

Ancestors of I.1 (Figure 2.3.2) can be traced back to a family of farmers living in rural Lincolnshire in the 16th century. Historical account from living and deceased family members and death certificates identify affected members with PD, back to the early 1800s.

Twenty-five affected members have been identified over four generations; only part of the pedigree is shown in Figure 2.3.2. At the time of the previous description of this kindred (Nicholl et al., 2002), there were five living subjects with PD (III.5, III.9, III.10, III.13, III.15) and sixth (IV.4) had just developed PD. At follow-up subject IV.4 had extrapyramidal signs and patients III.9 and III.13 had died; post-mortem examination was available on brain and olfactory bulb on patient III.13 (Khan et al., 2005). All clinical details are summarised in Table 2.3.2.

Patient I.1

This subject, born in the late 1800s, is reported to have developed tremor in his sixties. Several grandchildren have commented that this was relatively mild compared to subsequent generations. He died aged 67 years.

Patient II.1 and offspring III.2

II.1 is reported by family members to have had mild signs of parkinsonism in her early seventies and died in 1976, aged 85 years. One of her children, III.2 developed parkinsonism at the age of 48 years but died aged 57 from bronchopneumonia. A post-mortem was not performed on the brain.

Patient II.4 and offspring III.5

II.4 developed parkinsonism at 61 years. Other family members report the subsequent development of a shuffling gait, falls and severe parkinsonism within 5 years. He died aged 66 years from bronchopneumonia in 1969 and did not have a post mortem

Figure 2.3.2

Family tree of British kindred with Autosomal Dominant PD

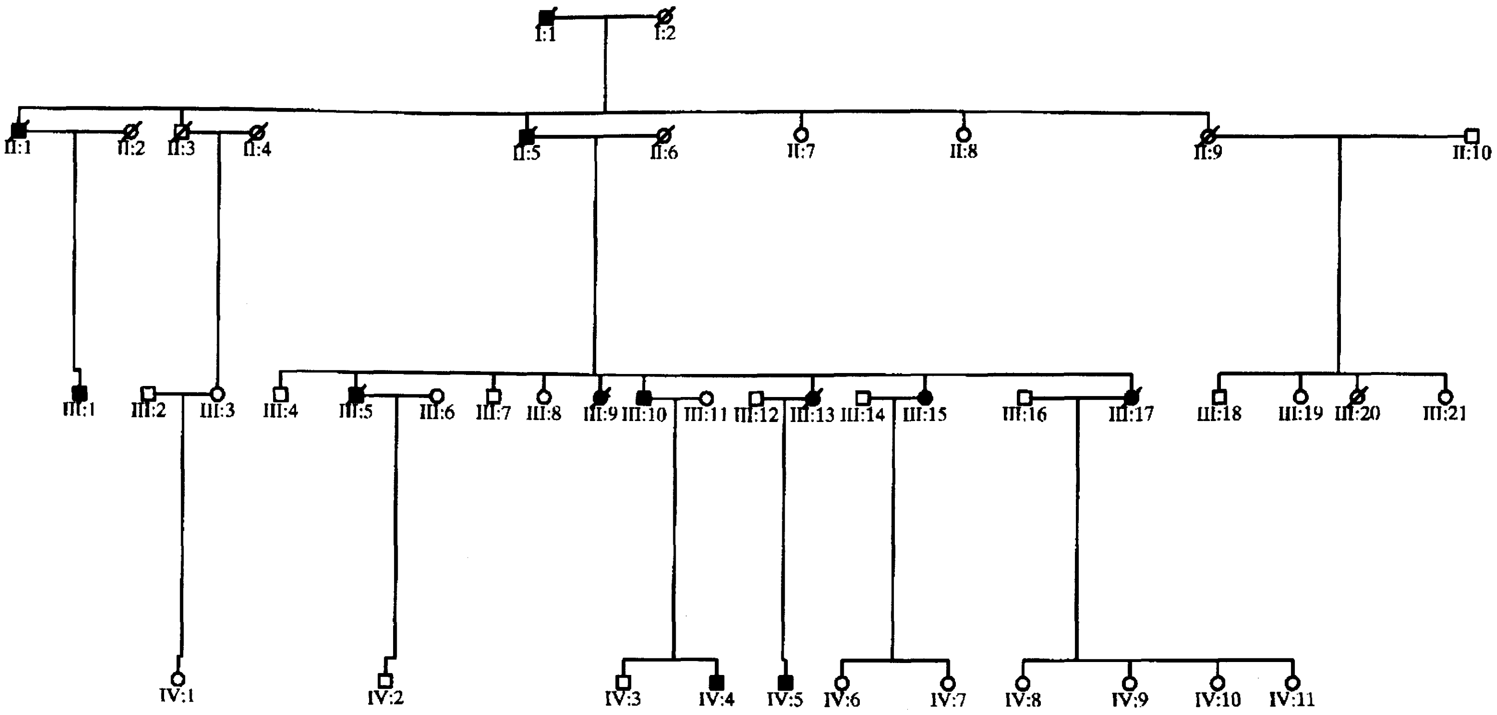

I:1
I:2
II:1
II:2
II:3
II:4
II:5
II:6
II:7
II:8
II:9
II:10
III:1
III:2
III:3
III:4
III:5
III:6
III:7
III:8
III:9
III:10
III:11
III:12
III:13
III:14
III:15
III:16
III:17
III:18
III:19
III:20
III:21
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
IV:7
IV:8
IV:9
IV:10
IV:11

Table 2.3.2 Clinical characteristics of affected subjects in the British kindred.

Subject	Sex/Age	Age at Onset	Symptoms at onset	DD	Response to L-dopa	Other features
I.1	M/67	65	tremor	5	not taken	b. late 1800's, 'mild' disease, died aged 67
II.1	M/66	61	unknown	5	not taken	b. 1890's, shuffling gait, severe parkinsonism, died aged 66
II.5	M/70	50	unknown	20	not taken	b. 1890's late disease: severe parkinsonism; died aged 70
II.9	F/85	75	unknown	15	unknown	b.1890's ; 'mild' disease, died aged 85
III.1	M/70	56	rest tremor big toe	14	excellent	anxiety at onset, foot dystonia & dyskinesia after 4yrs treatment, MMSE 30 at aged 70
III.5	M/54	50	unknown	5	good	10 years of anxiety and neurosis prior to the onset parkinsonism died aged 70
III.9	F/77	57	unknown	20	significant	after 10 yrs: severe offs, bulbar symptoms, akinetic & mute, died 77
III.10	M/70	45	unilateral leg tremor	25	significant	suicide attempt at 66, depression for ten years, continued response to L-dopa, no dyskinesias. MMSE 30
III.13	F/70	50	unilateral leg stiffness & tremor	20	moderate	after 10 years: depression, foot dystonia (rest / exercised induced), peak-dose dyskinesias; died aged 70 (stroke) PM +
III.15	F/68	44	unilateral leg tremor & stiffness	26	significant	claustrophobia, anxiety & depression after 14 yrs, MMSE 28 at 68 dyskinesias after 8 yrs
III.17	F/57	45	unilateral leg tremor & stiffness	12	significant	paranoia, anxiety & depression after 10 yrs, foot dystonia, little dyskinesias on treatment, died aged 57.
III.20	F/57	48	unknown	11	unknown	hemi-parkinsonism, died from bronchopneumonia. No PM
IV.4	M/44	47	unilateral leg tremor	4	unmedicated	most recent subject who has developed disease
IV.5	M/49	40	unilateral leg tremor	9	significant	anxiety two years prior to disease onset, foot dystonia before L-dopa MMSE 30

examination.

His only child, subject III.5, aged 70years, first reported symptoms at the age of 56 years. He described anxiety, rest tremor of the big toe on the right foot which progressed to rest tremor of both legs. One year after the onset of symptoms he was noted to have a paucity of facial expression and poor arm swing and commenced L-dopa reporting a 90% improvement. Four years after treatment, he developed peak dose dyskinesias and right-sided foot dystonia both at rest and with exercise. He reported one fall per month for the past six years and freezing episodes for the past four years. He did not report any symptoms of autonomic dysfunction. There is no history of sleep benefit, motor fluctuations prior to starting treatment or any form of behavioural disorder prior to the onset of, or with disease. MMSE score was 30 aged 70 years. This patient has previously had an ^{18}F-dopa PET scan (Nichol et al., 2003).

Patient II.5 and affected offspring (III.5, III.9, III.10, III.13, III.15, III.17)

II.5 developed parkinsonism aged 50. Family members report that late disease associated with severe disability, marked facial hypomimia, severe rest tremor, akinesia and rigidity. He died, aged 70 years in 1966.

Patient III.5

This patient had a longstanding history of anxiety and neurosis before the onset L-dopa responsive parkinsonism aged 50 years. He died aged 54 years old.

Patient III.9 developed hemi-parkinsonism at the age of 57; there was a definitive response to L-dopa but this was complicated by end of dose akinesia. After ten years disease duration, aged 67, there was severe on-off phenomena and difficulty with speech and swallowing when off. At that time, when 'off', she was mute, with left sided hemi-parkinsonism and some restriction of upgaze only. The disease became marked disabling requiring care in a nursing home for nine years until the patient died aged 77. A post-mortem examination was not possible.

Patient III.10 This 70year old man noticed tremor in the left leg, especially prominent when seated aged 45 years. At age of 50 years he developed stiffness of the left arm and leg, foot dystonia and poor swing on the left. Two years later he commenced L-dopa and reported a significant improvement. After 35 years of disease he continues to report improvement with L-dopa, has no dyskinesias, reports falls over the past 18 months and freezing over the past 12 months. He attempted suicide attempt aged 66 years and has been treated for depression since.

Patient III.13 developed parkinsonism aged 50 years, with dragging of the left leg and subsequent tremor of the left arm and leg. L-dopa commenced two years after symptom onset with some improvement of symptoms. Peak dose dyskinesias, foot dystonia (rest and exercise-induced) and depression occurred ten years later. Freezing, falls and postural-related symptoms occurred in the last five years of disease. The patient died, aged 70 years, in 2000 after a stroke. A post-mortem examination was performed and brain histopathology was studied by Dr Janice Holton, Consultant Pathologist, Institute of Neurology, Queen Square London. The report is discussed in the conclusion of this chapter.

Patient III.15 This 68 year old, reported both rest tremor and dragging of the left leg and foot at the age of 44 years. At that time, she commenced benzhexol and reported a significant improvement of symptoms. Within two years she developed a prominent tremor of the left hand, flexed posturing, poor arm swing and dystonia of the left foot at rest and with exercise. L-dopa was started 10 years after disease onset, on which she reported 80 % improvement of symptoms. Dyskinesias started after eight years of L-dopa therapy. Falls and freezing have complicated disease in the past year. There are no urinary or postural-related symptoms. She developed severe claustrophobia, panic attacks and depression fourteen years after disease onset. MMSE was 28 aged 68 years.

III.17 developed parkinsonism aged 45 years. At disease onset she would often trip over and began to drag the left foot and leg. She subsequently developed rest tremor in the left arm and significant left-sided foot dystonia. L-dopa therapy started one year after disease onset with significant improvement. Significant depression, paranoia and panic attacks occurred ten years after disease- onset. At age 53, a hemi-colectomy was performed after developing complications of chronic constipation. This subject had little dyskinesia and was predominantly akinetic and died after disease duration of 12 years.

Patient IV.4

This 47 year old (offspring of III.10), first noticed intermittent tremor and dragging of the left leg aged 44 years. Aged 45 years he has poor arm swing on left and stiffness of left arm and leg. He was reviewed by a local Neurologist in 2004 who confirmed the diagnosis of PD. This patient is unmedicated.

Patient IV.5

This 49year old patient (offspring of patient III.13) first noticed tremulousness of the right leg whilst driving aged 40 years. There was also heaviness of the right leg and intermittent slurring of speech. Three years later, following a fall, he developed intermittent, right-sided foot dystonia at rest. Initial treatment commenced aged 44 years wit ropinirole and then pramipexole on which he reported 75% improvement of symptoms. Three years after treatment he commenced L-dopa and described a significant improvement of symptoms. He reported no dyskinesias, falls or freezing episodes. He developed prominent panic attacks two years prior to the onset of disease.

Age of Onset

Mean age of onset of living subjects remained at 57 years as previously reported (Nicholl et al., 2002) however in this study the mean age of onset of disease, decreased in successive generations; generation II, 62.0 years, generation III, 50.1 years and generation IV, 40 years.

Tremor

All subjects that were examined reported initial unilateral leg symptoms at onset. Subject III.5 aged 50, reported rest tremor of the big toe of the right foot that progressed to both feet and thereafter both legs. Subject III.10 reported an initial symptom of unilateral tremor in one leg that was prominent only when seated. IV.5 first noticed tremor and heaviness in the right leg that initially only occurred whilst driving. Subject III.17 first noticed tripping over her left foot aged 45 years. In addition she would also intermittently drag her left foot and leg; she subsequently developed rest tremor in left arm and left sided foot dystonia

Dystonia

Foot dystonia (rest and exercised-induced) was reported prior to and during L-dopa treatment; the latter developed early or later on in disease.

Other motor features

All subjects interviewed reported hemi-parkinsonian symptoms at onset with an overall picture of slowly progressive parkinsonism. Freezing episodes and falls occurred late in disease. Only one subject (III.9) reported significant bulbar symptoms late in disease.

Autonomic symptoms

Only one subject (III.13) reported symptoms of orthostatic hypotension after 15 years of disease.

Cognitive function

Cognition in subjects examined in detail (III.1, III.10, III.15, IV.5) was normal (MMSE >28). In particular subject III.15 was normal despite disease duration of up to 26 years.

Response to treatment

Disease in this kindred was characterised by significant response to L-dopa; subject III.10 reported sustained improvement even after twenty-five years of disease. In addition this subject did not report dyskinesias despite 18 years of L-dopa treatment. Other subjects reported little or no dyskinesia and mild foot dystonia that was more prominent at peak dose of L-dopa medication. Subject IV.5 reported significant benefit from ropinirole and pramipexole as first line treatment that was started 4 years after disease onset. He required L-Dopa three years later. Subject III.15 reported significant improvement on benzhexol for ten years and continued to report sustained improvement after substitution with L-dopa treatment.

Behavioural disorder

Seven subjects reported behavioural disorder (anxiety attacks, depression, paranoia and suicide): six reported symptoms after the onset of parkinsonism and subject IV.5 described symptoms two years prior to disease onset.

Olfaction testing

Patients III.1, III.10, III.15, and IV.5 had scores of 34, 24, 25 and 36 out of 40 respectively on UPSIT testing.

Unaffected subjects.

Subjects IV.3 (aged 42 years) and IV.6 (aged 48 years) declined a neurological examination or a PET scan. They were asymptomatic and are reported not to have signs of parkinsonism. Subjects III.3 (aged 77), III.4 (aged 83), III.7 (aged 78), III.21 (aged 82), IV.7 (aged 42) had normal examinations, Subject II.7 was aged 103 years is not reported to have developed parkinsonism.

2.4 Conclusion

This is the first detailed clinical report of the British kindred with ADPD; disease progression and duration was similar to Parkinson's disease. This is also the first detailed report of a kindred with a LRRK2 mutation.

The phenotype of the British kindred from Lincolnshire however is similar to the Sagamihara kindred that was the first to be linked to PARK8 (Funayama et al., 2002) the North American kindred (family D, Zimprich et al., 2004) and is not dissimilar to idiopathic Parkinson's disease. The mean age of onset in the Japanese, North American and British kindreds was 51, 65, and 57years respectively. In addition the age of onset of disease in the British kindred appeared to decrease by a decade in successive generations however this may reflect ascertainment bias.

Clinical features common to all three PARK8 kindreds included unilateral signs at onset and L-dopa responsiveness. In addition a more detailed study of the British kindred noted a sustained response to L-dopa despite years of disease and treatment; subject III.10 after twenty-five years of disease and eighteen years of L-dopa, continued to benefit without dyskinesias. Subject III.15 reported a significant improvement on benzhexol started at disease onset and continued response thereafter for ten years. Subject IV.5, with age of onset aged 40 years described significant symptom benefit from dopamine agonists for at least two years and thereafter a sustained response to L-dopa. Overall, patients did not report troublesome dyskinesias complicating treatment. Prominent foot dystonia (at rest and exercise induced) prior to and during drug treatment analogous to that reported in parkin disease was also noted (Khan et al., 2003).

Unilateral symptom onset of tremor in the foot or leg was reported by most subjects in this kindred. Prominent symptoms of tremor has also been observed in the Basque kindreds in which the PARK8 gene was originally cloned and it is for this reason that the protein has been named 'dardara' derived from the Basque word 'dardara' meaning 'tremor' (Paisan-Ruiz et al., 2004).

Cognition in this kindred was not significantly abnormal despite a lengthy disease duration (up to twenty six years) in some subjects (Table 2.3.2); this may suggest that the neurodegenerative pathway involving aberrant LRRK2 product may spare structures critical to cognitive function. Functional abnormalities in the caudate nucleus have been postulated to play a role in frontal and subcortical dementia in patients with basal ganglia pathology.

Behavioural disorder was noted in a number of affected subjects. Psychiatric complications including depression, anxiety and psychosis have been reported in IPD with rates for depression ranging from 20-50% (Oertel et al., 2001), anxiety disorders from 20-40% and psychosis from 15-20% (Aarsland et al., 1999). In this kindred it was observed in patients with a younger age of onset (mostly <50 years) analagous to findings of behavioural disorder in parkin disease (Khan et al., 2003).

The phenotype of the other autosomal dominant loci differs somewhat from idiopathic Parkinson's disease and PARK8; the clinical characteristics of patients with the A53T mutation in the α-synuclein gene have a lower age of onset (45 years), a much lower prevalence of tremor, more rapid clinical deterioration as well as dementia and hypoventilation (Polymeropolus et al., 1997, Papapetropoulos et al., 2001). PARK3 families have an age of onset that can be as low as 37 years with a phenotype that encompasses significant cognitive impairment.

Olfactory dysfunction is found in 70-100% of PD patients rendering it as common a clinical sign as pill rolling tremor (Doty et al., 1988, Katzenschlager et al., 2004). The exact pathogenesis of olfactory dysfunction is unclear however the detection of neuronal loss with Lewy body deposition in the olfactory pathway in PD (Daniel et al., 1992) suggests that, at least in this disease, the mechanism is central and neurodegenerative. More recently dopamine levels have been reported to be increased in the olfactory bulb in PD (Huisman at al., 2004) and animal data suggests that dopamine has an inhibitory effect on transmission in the olfactory pathway (Katzenschlager et al., 2004). Lewy bodies have been identified in the olfactory bulb in patient III.13 (Figure 2.4a) (Khan et al., 2005) implying that altered kinase activation by mutant LRRK2 protein extends to the olfactory neural networks. Olfaction in four of the affected subjects however did not uniformly detect anosmia, despite lengthy disease duration in these subjects. Although the number of patients in whom olfaction could be tested was too small for meaningful statistical analysis, their mean score was 29.7 which was similar to normal, age-matched British controls with a mean of 27.6 (Katzenschlager et al., 2004). This is in contrast to the cohort of unrelated British IPD patients with a mean UPSIT score of 17.1 (Katzenschlager et al., 2004). Discordance between histopathological findings of Lewy bodies in the olfactory bulb of subject III.13 (Khan et al., 2005) and a normal mean UPSIT score in other affecteds may be explained by the fact that up to 30 % of PD patients have normal smell despite Lewy body disease (Katzenschlager et al., 2004) or, that the Lewy body load in the olfactory pathway is not as deleterious in LRRK2-associated PD. In addition we were unable to perform a direct correlation of pathology with UPSIT score; the latter was not available on subject III.13. Higher UPSIT scores have been documented in tremor-dominant PD patients with a family history of tremor (Ondo et al., 2003). Further studies of a much larger, unrelated cohort will be necessary to assess olfaction in PARK2 / LRRK2 patients.

Pathology in this British kindred is associated with typical Lewy bodies with marked loss of pigmented neurons and gliosis of the substantia nigra with small numbers of cortical and brainstem Lewy bodies (Figure 2.4a) (Khan et al., 2005). Moreover this complements *in vivo* functional imaging performed on another affected subject, patient III.1 in whom ^{18}F-dopa PET findings showed pattern of nigrostriatal dysfunction typically seen in PD (Nicholl et al., 2002) (Figure 2.4b); an anteroposterior gradient of putamen tracer distribution reflecting the preferential degeneration of the ventrolateral tier of the substantia nigra pars compacta which projects to the posterior dorsal putamen with relative sparing of the dorsomedial nigral cells which project to the anterior dorsal putamen and head of the caudate (Bernheimer et al., 1973). More recent ^{18}Fdopa PET studies in patients with LRRK2 mutations have confirmed this (Adams et al., 2005, Hernandez et al., 2005). A similar pattern of nigrostriatal dysfunction has also been observed in four PD patients from the Greek-American kindred with dominant disease with a G209A mutation in alpha synuclein (Samii et al., 1999) and contrasts other forms of familial parkinsonism including some patients with parkin disease (PARK2) and early onset parkinsonism associated with mutations in PINK1 (PARK6) where there is more uniform loss of striatal dopamine terminal function with greater involvement of head of caudate and anterior putamen compared to PD (Khan et al., 2002).

Pathology that is typical of PD has also been reported in other PARK8 –linked kindreds (Zimprich et al., 2004b, Wszolek et al., 2004) however in the original description of the Japanese kindred, the first to be linked to PARK8, the pathological examination was remarkable for showing nigral degeneration without Lewy bodies (Funayama et al., 2002). Two other North American kindreds with LRRK2 mutations 2004b) has additional clinical and pathological features consistent with anterior horn cell degeneration. It is interesting that this family shares the same LRRK2 mutation with the British kindred (personal communication T Gasser) in whom the phenotype,

Figure 2.4a. Histopathology of patient III.13 (Figure 2.3.2). In the substantia nigra there was very severe loss of pigmented neurons with small numbers of Lewy bodies (arrow and insert A). Lewy bodies (B) and Lewy neurites (C) were identified in the substantia nigra using alpha-synuclein immunohistochemistry. Similarly there were small numbers of Lewy neurites (D) and occasional Lewy bodies (inset in D) in the olfactory bulb. Lewy bodies were scanty in the neocortex (E). Bar represents 28μm in A-E and 18μm in the insert in A. A: haematoxylin and eosin; B-E: alpha-synuclein immunohistochemistry. With kind permission from Dr Janice Holton, Institute of Neurology, Queen Square, London (Khan et al, 2005).

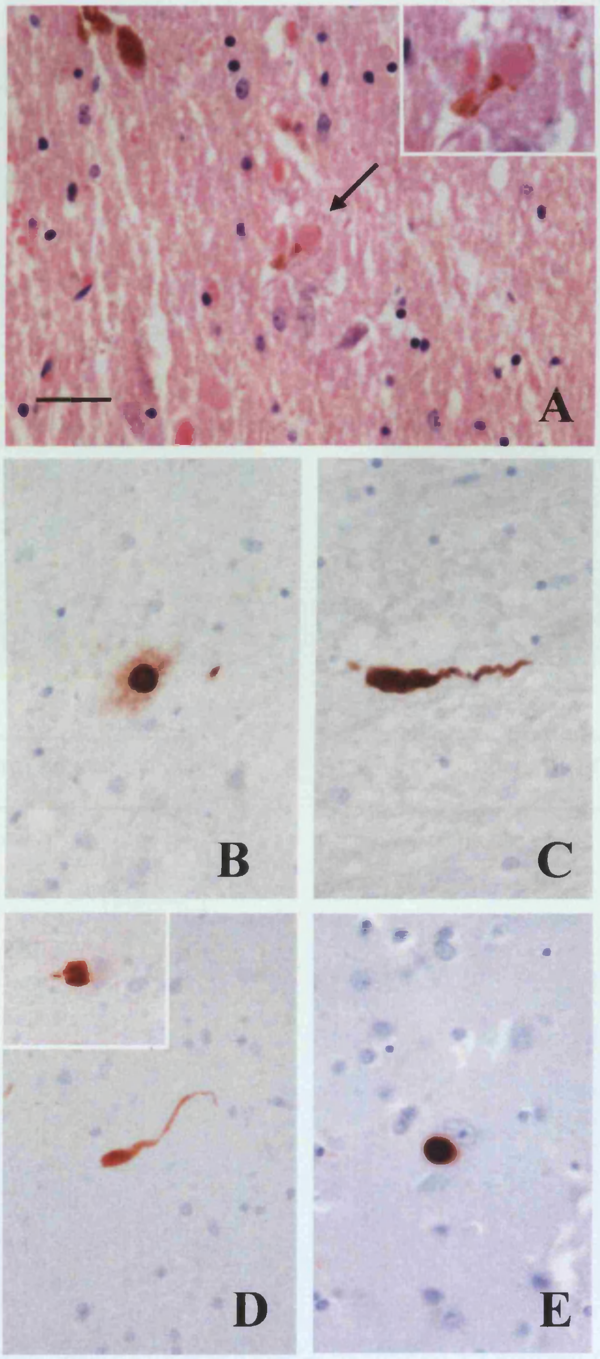
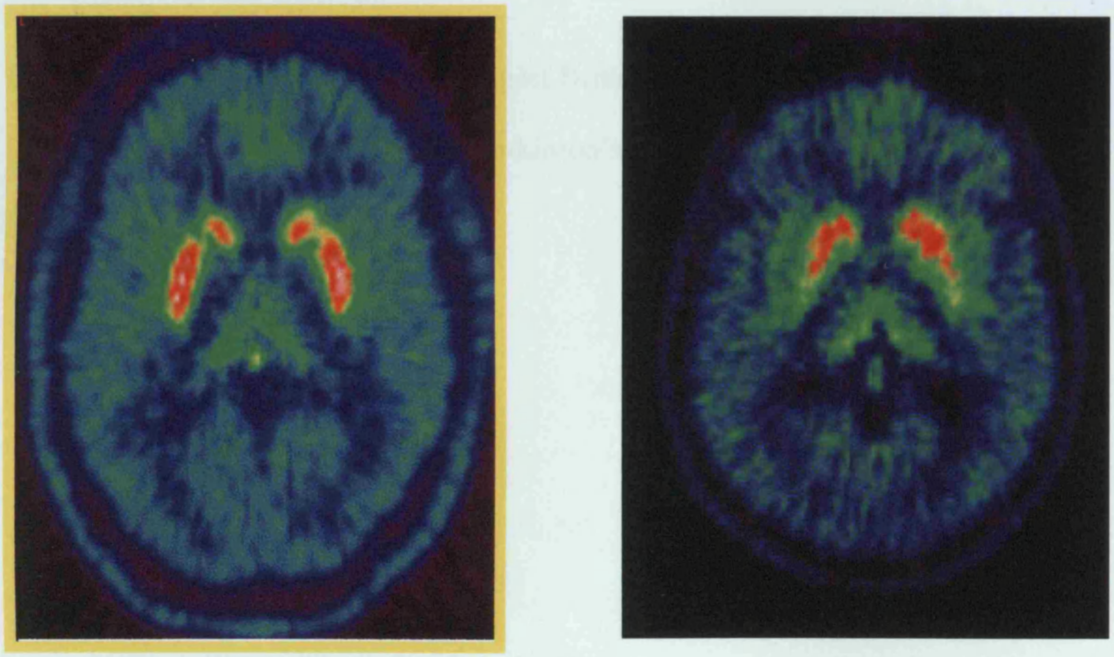


Figure 2.4b. ^{18}F-dopa PET scans showing nigrostriatal dysfunction in patient III.1 (Figure 2.3.2) and a healthy control. With kind permission from Dr Paola Piccini, Clinical Sciences Centre, MRC Cyclotron unit, Hammersmith Hospital, London



Control subject

Patient III.1 (Figure 2.3.2)

pathology and functional imaging that is strikingly similar to IPD. One explanation for discordance between genotype and phenotype in the British kindred and North American Family A may be that two separate diseases and therefore two distinct types of pathology exist in the latter. A study, of both phenotype and pathology in a larger number of patients sharing the same LRRK2 mutation will be necessary to confirm this.

In conclusion, the phenotype of the largest British kindred with autosomal dominant PD is similar to idiopathic, late-onset Parkinson's disease.

3. THE GENETIC CHARACTERISATION OF THE LARGEST BRITISH KINDRED WITH AUTOSOMAL DOMINANT PARKINSON'S DISEASE.

3.1 Introduction

Autosomal dominant Parkinson's disease is genetically heterogeneous. Prior to this study mutations in the coding regions of *SNCA* and *UCHL1* as well as linkage of the disease to a haplotype on chromosome 2p13 and chromosome 4p (previously designated PARK4) had been excluded in this kindred (screened by Dr Jenny Vaughan, Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London; Nichol et al., 2002) thus providing further evidence for locus heterogeneity in ADPD. In addition one affected (patient III.1, Figure 2.3.2) did not have a mutation in HD, DRPLA and SCA3 (screened by Victoria Stinton and Tunde Akimbode of the Diagnostic Laboratory, Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London).

The aim of this study was to map a novel locus using a genome-wide search in the British Kindred with ADPD whose phenotype is reported in the chapter two.

3.2 Methods

3.2.1 General Methods

3.2.1.1 Patients

All subjects gave informed consent and the study was approved by the Ethics Committees of the National Hospital for Neurology and Neurosurgery.

3.2.1.2 Blood Collection

Peripheral blood was re-collected from six affected subjects with L-dopa responsive parkinsonism and nineteen unaffected, at risk members and two spouses. Blood was sampled from the antecubital vein in vacutainers containing tripotassium EDTA. Samples were frozen and stored for future genomic DNA extraction.

3.2.1.3 Genomic DNA extraction

Genomic DNA was extracted from peripheral blood leucocytes using a semi-automated phenol-chloroform method. Genomic DNA was extracted from 10ml venous blood collected in EDTA blood tubes. The sample was split into 2x 50ml falcon tubes, and 40ml Reagent A was added to lyse the red blood cells. The samples were spun in a Beckman centrifuge (model CS-6R) at 2600rpm for 20 minutes. The supernatant was discarded and the pellet washed with 5ml PBS. After vortexing to resuspend the pelleted cells, the sample was centrifuged again at 2600 rpm for 8 minutes. The supernatant was discarded and the pellet washed again with 5 ml PBS. After vortexing again, the sample was spun again at 2600 rpm for 8 minutes. The supernatant was discarded, and 500 µL of a solution containing 25µl 10% SDS, 500µl CVS buffer and 100µl proteinase K solution was added to the pelleted cells and the sample was placed in an incubator at 55C for overnight digestion. Each sample was phenol-chloroform extracted to remove proteins. 5ml phenol was added to each pellet (10mM Tris, pH 8, 1mM EDTA, inverted to mix sample and centrifuged at 2600 rpm for 15 minutes. The top aqueous layer was removed and placed in a clean tube, and a chloroform extraction step was performed twice, by adding 5 ml chloroform / isoamyl alcohol (24:1) shaking well to mix, and spinning at 2600 rpm for 15 minutes to remove the phenol. DNA was precipitated by adding15ml 100% ethanol (see section 3.2.12) and hooking the DNA threads out with a pipette tip. The DNA was re-suspended in 300 µl 1x TE and left to dissolve at room temperature overnight.

3.2.1.4 Quantification of DNA

The concentration of DNA in aqueous solutions was determined by measuring the optical density (OD) of the sample at wavelengths 260 nm and 280 nm in the UV spectrum using a spectrophotometer (RNA/DNA Calculator Genequant, Pharmacia

Biotech). 5µl DNA was diluted in 995µl distilled water and placed in a quartz cuvette. The OD was measured against a "blank" sample (1000µl distilled water) and the concentration determined. An OD of 1.0 at 260 nm corresponds to 50 mg/ml double stranded DNA or 20 mg/ml oligonucleotide. Therefore the concentration of a DNA sample (in mg/ml) is calculated by: $OD^{260\text{ nm}}$ x conversion factor (50) x dilution factor (200)

The ratio of the OD readings at 260 nm/280 nm provides an assessment of the purity of the DNA sample with pure samples having a ratio of greater than 1.8. A ratio of less than 1.5 indicates that the sample was contaminated with proteins and needed an extra extraction with phenol chloroform.

3.2.1.5 Amplification of DNA fragments by polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) allows the amplification of specific DNA regions that lie between two regions of known sequence. The reaction is initiated using oligonucleotide primers which are short (18-20bp) single stranded DNA molecules complimentary to the ends of the defined sequences of DNA template. The double stranded template is separated into single strands by thermal denaturation, then cooled to allow sequence specific annealing of primers. Annealing of primers is favoured rather than reannealing of template strand is favoured by the high concentration of the primers relative to the template. The primers were extended on a single stranded template by a thermostable DNA polymerase in the presence of deoxynucleoside triphosphates (dNTPs), under suitable reaction conditions. This results in the synthesis of new DNA strands complementary to both strands of the template. The process is repeated, and each newly synthesized strand becomes a template, the process leading to an exponential amplification of the target sequence.

PCR reactions using fluorescently labelled primers were usually carried out in final reaction volumes of 7.5 µl in 96-well microtitre plates (Micro Test III, Falcon). The reaction mixture consisted of: 0.2 mM each of dATP, dCTP, dGTP and dTTP (0.94 µl of 10 x dNTP solution, Promega); 0.75 µl of GeneAmp 10 x magnesium-free PCR Buffer II (Perkin Elmer); 1.5 mM $MgCl_2$ (0.75 µl of 25 mM $MgCl_2$ solution, Perkin-Elmer); 0.5 µl of each primer; autoclaved and filtered distilled water to make up reaction mixture volume to 7.5 µl; 0.3 units of DNA polymerase added last (AmpliTaq Gold^{TM} 5units/µl, Perkin-Elmer). The reaction mixture was prepared at room temperature and aliquoted into microtitre plate wells using an eight channel pipette (Scotlab). 25 ng (2.5 µl) of template DNA was then added to each well. Microtitre plates were then centrifuged at 1000 rpm for 30 seconds (Beckman GS-6R centrifuge).

The microsatellite markers from the Linkage Mapping Set ABI PRISM LD-10 were used for genome-wide searches two primers from the same panel were multiplexed together in the same PCR reaction, to save time and consumables. PCR conditions for these markers have been optimised by the manufacturers, and all markers in the set can be PCR-amplified under the same conditions. PCR reactions were performed using a Perkin Elmer 9700 thermal cycler. Reaction mixes were first heated to 95^0C for 11 minutes to activate the AmpliTaq Gold^{TM}; subsequent cycling conditions were 94^0C (denaturation) 30 seconds, 60^0C (annealing) 15 seconds, 72^0C (extension) 15 seconds; repeated for 20 cycles, then: 94^0C (denaturation) 30 seconds, 55^0C (annealing) 15 seconds, 72^0C (extension) 15 seconds; repeated for 15 cycles.

All reagents and materials used were sterile and a negative control (omitting template DNA) was always included in experiments.

3.2.1.6 Oligonucleotide primers for microsatellite markers.

For the genome-wide search a set of fluorescently labelled primers were used. Genotyping was performed using 382 microsatellite markers (fluorescent, highly informative $(CA)_n$ repeat microsatellite markers located at average intervals of 10cM) from the ABI PRISM Linkage Mapping Set (LMS) Version 2 (Foster Kennedy, CA, USA).

The markers are arranged into 28 panels containing 10 to 20 fluorescent dye-labelled primer pairs (6-FAM, NED or HEX) that generate PCR products that can be combined and detected in a single gel lane. Forward and reverse primers are combined and supplied in a tube at 10µM concentration (5 µM of each primer) in 10mM Tris-HCl, 1 mM EDTA, pH 8.0.

Additional microsatellite markers were used to narrow the region identified were analysed using custom-made fluorescently labelled primers. Primers were manufactured by Perkin-Elmer with a 5' 6-FAM, HEX or TET (or NED) dye on one of each primer pair. Markers used were oligonucleotides from the Marshfield Centre for Medical Genetics Human Genetic Map (Sheffield et al, 1995; http://www.marshfieldclinic.org). In all cases, markers with the highest heterozygosity were selected for use in order to maximise informativeness.

3.2.1.7 Agarose Gel Electrophoresis.

To check for the presence of a PCR product of the desired size and quantity, 2 µl aliquots of reaction mix from four randomly selected wells (as well as the negative control) were visualised by electrophoresis on ethidium bromide stained agarose gels. 5 µl of reaction mix was added to 2 µl of agarose gel loading buffer (6x buffer consists of 40% w/v sucrose, 0.25% bromophenol blue) and electrophoresed at 50 V through a 3.2% agarose minigel (Flowgen Instruments Ltd) for 30 - 60 minutes. A

100-bp size standard (Gibco) (1 μl) was run alongside the PCR products to enable estimation of their size. Ethidium bromide staining of the agarose gel (1 mg / ml, Sigma) permitted direct visualisation of DNA products using transillumination with ultraviolet light.

3.2.1.8 Polyacrylamide gel electrophoresis

Electrophoresis through a polyacrylamide gel is a means of separating small DNA fragments with high resolution, allowing fragments differing in size of 1-bp to be separated. Denaturing polyacrylamide gels are polymerised in the presence of an agent such as urea which suppresses base pairing in nucleic acids. Denatured (single stranded) DNA migrates through these gels at a rate that is determined by fragment size and almost completely independent of base sequence and composition, permitting sizing of fragments according to distance travelled through the gel (smaller fragments migrate further than larger ones).

PCR products produced using fluorescently tagged primers were sized by electrophoresis through a denaturing 4 % polyacrylamide gel in an automated DNA sequencer (Applied Biosystems, model 377). During electrophoresis, a section of the gel furthest from the loading comb was scanned by a laser causing each dye moiety (attached to one oligonucleotide primer incorporated into PCR fragments) to emit light of a known wavelength as it migrates past the laser. A size standard consisting of DNA fragments of known size, labelled with the fluorescent dye TAMRA or ROX, is run in each lane to allow accurate sizing of PCR fragments. This method of DNA sizing has the great advantage over radioactive methods that markers of non-overlapping size and dye composition may be multiplexed in each lane so maximising efficiency and increasing sample throughput. As many as 24 microsatellites may be run in each lane although in practice a maximum of 20 markers were run simultaneously during this study.

3.2.1.9 Polyacrylamide gel preparation.

Thirty-six cm well-to-read glass plates were used with a 377 sequencer. Plates were cleaned with detergent and rinsed with distilled water. The dry plates were assembled in the 377 cassette prior to pouring the gel. The catalysts TEMED (Sigma) (35 μl) and freshly prepared 10% ammonium persulphate solution (APS) (Sigma) (250 μl) were added to 50 ml of 4% acrylamide gel mix to start polymerisation. The mix was then taken up into a 50 ml syringe and carefully introduced into the notch between the front and back plates, spreading evenly between the glass plates. A spacer was inserted into the upper notch between the plates and the gel left for two hours to polymerise. After polymerisation, the upper spacer was removed and a 48 or 64 well shark's tooth comb was carefully inserted in its place. The cassette and plates were then placed in the 377 sequencer and the plates checked for background fluorescence using Genescan software (Applied Biosystems). Heating plate and buffer chambers were assembled and 1.3 L of 1 x TBE buffer added before pre-running the sequencer until the gel temperature reached 50^0C. Samples were then loaded. For preparation of acrylamide gel mix and of TBE buffer, see paragraph "Buffers and solutions" at the end of this chapter.

3.2.1.10 Pooling of PCR products for loading.

Up to 20 non-overlapping microsatellite markers, amplified from a single DNA sample, were run simultaneously in each lane (multiplexed). PCR products from each DNA sample were first pooled according to the dye they contained as follows: FAM – 2.5 μl; TET - 5 μl (or NED - 5 μ); HEX - 5 μl. These volumes were adjusted according to the yield of the PCR as determined on agarose gel electrophoresis.

Pooling was performed in microtitre plates using an eight-channel pipette. 2.5 μl of pooled product from each well was then aliquoted into a fresh microtitre plate and an equal volume of loading mix added. The loading mix consisted of 100 μl of deionised formamide, 20 μl of loading buffer (blue dextran, 50mg/ml, EDTA 25 mM, Perkin Elmer) and 24 μl of Genescan 350-TAMRA or Genescan 400-HD ROX size standard (Applied Biosystems). The final mix of pooled product and loading mix was denatured at 95^0C for 2 minutes in a Hybaid thermal cycler and then placed immediately onto ice before loading.

3.2.1.11 Gel loading and electrophoresis conditions

Wells were carefully flushed with 1 x TBE buffer immediately prior to loading. Alternate (odd-numbered) wells were loaded with 1.0 μl of final mix using a P2 pipette (Gilson) and Sorenson MiniFlex 0.2 mm flat tips (Anachem). Great care was taken to avoid spillover into adjacent wells. Electrophoresis at 3,000 V for two minutes ensured that samples were run into the gel before even-numbered lanes were loaded. Loading of alternate lanes made it possible to distinguish adjacent lanes in the final gel image and improved the ability of the software to track lanes correctly. Total run time was two hours. A maximum of 48 or 64 samples could be run in adjacent lanes.

Amplified products were separated by electrophoresis using a denaturing polyacrylamide gel on an ABI PRISM 377 DNA sequencer (PE Applied Biosystems).

3.2.1.12 Data analysis for fluorescently labelled PCR products

Data collected during the electrophoresis run were analysed automatically using the Genescan software; DNA fragment size and analysis was performed using GENESCAN v3.1.2. Automatic lane tracking was checked using the gel image, and adjusted lane by lane where necessary. To ensure accurate sizing, the automatic designation of peak sizes for internal lane size standards was manually checked in each lane. Markers were only sized if there were two size standard bands of greater size, and two of smaller size, present in the lane.

3.2.2 Genotyping

Fragment size data collected using the Genescan software were then analysed using the Genotyper software; GENOTYPER v2.5.1 software (PE Applied Biosystems). The Genotyper software labels fluorescent peaks with fragment size (to 0.01 of a base) and filters out background peaks. Manual adjustments are required by scrolling through all electropherograms to ensure that alleles are correctly labelled. Although time-consuming, this step is extremely important as labelling of incorrect (non-allele) peaks is a major source of genotyping error if not manually checked. Peaks in each marker range are grouped into discrete alleles and sequentially numbered from smallest to largest. Genotypes were scored blind without reference to the family pedigree to minimise bias.

3.2.3 Linkage analysis

Initial two-point parametric linkage analysis was performed using MLINK programme of the FASTLINK package (Lathop et al., 1984, Lathop et al., 1985). The disease was assumed to be autosomal dominant with a gene frequency of 0.0001 with equal marker allele frequencies. There were three liability classes: I (affected), II (unaffected at 50% risk) and III (no risk of disease).

All linkage programs were remotely accessed through the Human Genome Mapping Project web site (http://www.hgmp.mrc.ac.uk). An affecteds-only methodology was used for the first screening during the genome-wide search, in order to avoid biases resulting from inclusion of possibly affected individuals or incorrect estimation of penetrance or age of onset. In a powerful enough family, linkage analysis with affected individuals only allows the exclusion of a large part of the genome (70-80%). The regions surrounding markers showing possible linkage with the disease (pairwise LOD score > 1) and all regions surrounding non-informative markers were always saturated with more markers and all available family members were genotyped in order to allow haplotype construction.

3.2.15 Haplotype Analysis

Haplotypes were manually constructed.

3.3 Results

3.3.1 Haplotype and Linkage analysis

From the genome screen a haplotype segregated in all clinically affected subjects between ABI LMS markers D12S99 to D12S83; D12S364 gave the highest LOD score of 3.55 (θ=0.00) (Table 3.3.1). D12S364 lies approximately 23-25cM telomeric of the PARK8 locus (PARK8 locus highlighted in bold in Table 3.3.1). Additional markers were genotyped to determine if this locus was independent of the PARK8 locus: D12S77, D12S62, D12S1631, D12S1653, D12S339 (Table 3.3.1 & Figure 3.3.1); this did not refine the region further (Figure 3.3.1). The only recombinants observed were in two unaffected subjects IV.3 aged 42 years and IV.7 aged 48 years (Figure 3.3.1 and Table 3.3.1). Elsewhere positive LOD scores (maximum LOD score >1 at θ=0.00) were generated on chromosome 3 and 21 however haplotype analysis formally excluded these as candidate regions.

3.4 Conclusion

This study identified a 50 cM disease-causing locus on the short arm of chromosome 12 in the British kindred with ADPD. During the laboratory work, Japanese colleagues established linkage to a 12cM region (encompassed within the region

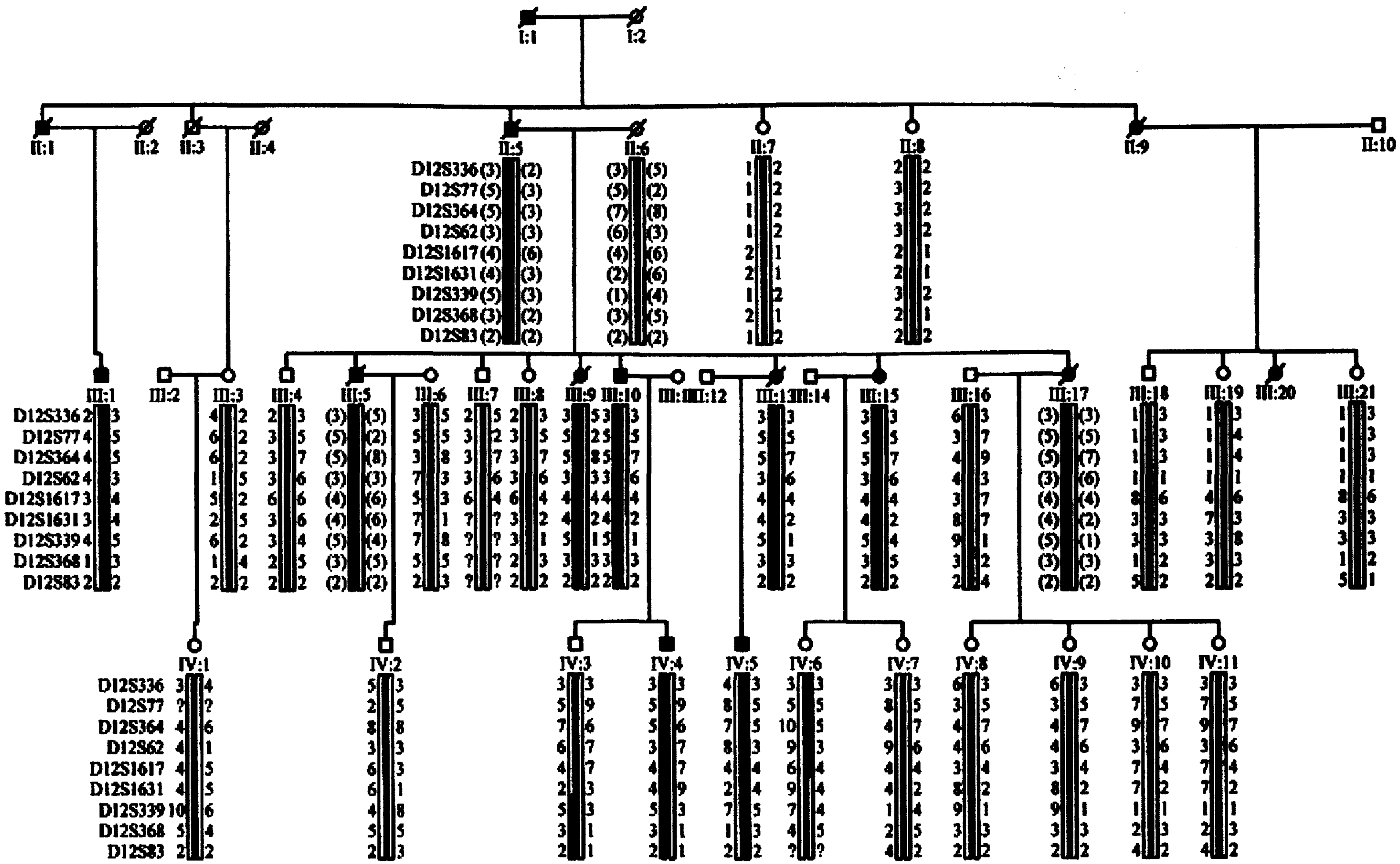
Table 3.3.1

Two-Point Linkage LOD scores between autosomal dominant PD and microsatellites from the genome screen on chromosome 12 and those in the original PARK8 region (highlighted in bold). The position of markers on chromosome 12 and genetic distances between markers were obtained from the deCODE linkage map.

		LOD Scores at recombination fraction θ						
Markers	Chromosomal position (cM)	0.00	0.01	0.05	0.1	0.2	0.3	0.4
D12S99	15.20	-infinity	-0.91	-0.19	0.07	0.19	0.11	0.01
D12S336	24.04	2.20	2.16	1.99	1.76	1.29	0.78	0.28
D12S77	24.85	2.93	2.88	2.65	2.36	1.74	1.08	0.4
D12S364	31.16	3.55	3.48	3.22	2.88	2.15	1.36	0.56
D12S62	32.48	1.48	1.44	1.29	1.10	0.72	0.39	0.14
D12S1617	45.91	2.45	2.40	2.21	1.97	1.44	0.88	0.31
D12S1631	50.90	1.92	1.87	1.64	1.34	0.77	0.30	0.04
D12S345	55.25	2.69	2.64	2.44	2.17	1.59	0.99	0.39
D12S339	65.8	3.42	3.36	3.08	2.73	1.97	1.16	0.35
D12S368	66.55	2.53	2.48	2.29	2.03	1.48	0.89	0.32
D12S83	74.03	0.23	0.22	0.19	0.16	0.09	0.04	0.01
D12S326	91.20	-2.62	-0.83	-0.26	-0.06	0.05	0.05	0.02

Figure 1

Haplotype Analysis of British kindred with mutations in LRRK2.


I:1
I:2
II:1
II:2
II:3
II:4
II:5
II:6
II:7
II:8
II:9
II:10
D12S336
D12S77
D12S364
D12S62
D12S1617
D12S1631
D12S339
D12S368
D12S83
III:1
III:2
III:3
III:4
III:5
III:6
III:7
III:8
III:9
III:10
III:11
III:12
III:13
III:14
III:15
III:16
III:17
III:18
III:19
III:20
III:21
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
IV:7
IV:8
IV:9
IV:10
IV:11

linked to ADPD in the British kindred) and designated the locus PARK8 (Funayama et al., 2002). Thereafter a North American kindred was also linked to the same locus (Family D, Zimprich et al., 2004). This confirmed not only genetic heterogeneity within ADPD but also suggested that mutations in PARK8 were unlikely to be a founder effect but were to represent a more common cause of familial, late onset PD; the British kindred originates from Lincolnshire, England and is unlikely to be related to the family originally described in Japan (Funayama et al., 2002) or to that in North America (Family D, Zimprich et al., 2004).

Subsequent work by colleague Shushant Jain, Institute of Neurology and collaborators in National Institute of Aging, National Institute of Health, USA (C Paisan-Ruiz and Dr Andrew Singleton) refined the disease interval further following the identification of a common haplotype in four Basque families (Paisan-Ruiz et al., 2004). This enabled a candidate gene sequencing strategy to be undertaken. Subsequently, disease-causing mutations were identified in a novel gene, LRRK2 (leucine-rich repeat kinase 2) (MIM 607060) in this British kindred and the Basque kindreds (Paisan-Ruiz et al., 2004).

The LRRK2 gene has 51 exons, encoding a product with 2000 amino acids constituting a large, multifunctional protein belonging to the ROCO protein family.

A Y1699C in exon 35, in LRRK2 has been identified only in clinically affected members in the British kindred (Paisan Ruiz et al., 2004). The disease in this kindred appears fully penetrant implying that other susceptibility loci and/or undefined environmental factors are not necessary for disease expression. Incomplete penetrance however has been reported in the original PARK8 kindred (Funayama et al., 2002) and confirmed in other European kindreds with LRRK2 mutations; this appears to be age dependent being 17% at 50years and 85% at 70years (Kachergus et al., 2005).

Moreover a recent case has been described of an octogenerian with a G2019S mutation who has an entirely normal examination (Kay et al., 2005).

Separate founder effects have been reported. The first a region of Northern Spain that geographically lies adjacent to the Basque region. Here 2.7% of a cohort of 225 PD patients had a R1441G mutation and all shared a common haplotype (Mata et al., 2005). In a second cohort of 248 patients, 2.8% carried a G2019S mutation in exon 41; all shared a common European ancestral haplotype in patients from the USA, Ireland and Poland amongst others (Kachergus et al., 2005).

Mutations in the gene encoding the LRRK2 protein will prove to have a numerically greater contribution to the aetiology of late-onset, familial Parkinson's disease, than other dominant loci identified to date.

4. CLINICAL CHARACTERISIATION OF EARLY ONSET PARKINSONISM DUE TO MUTATIONS IN PARK2 (PARKIN DISEASE).

4.1 Introduction

At the time of this study there was a wealth of molecular genetic data with little detail of the clinical phenotype of EOPD with mutations in PARK2. This chapter reports the clinical characterisation of parkin disease and is divided into two parts:

i) A phenotypic study of a large case series with parkin disease.

ii) Olfactory function in parkin disease compared to early-onset parkinsonism without parkin mutations and to idiopathic Parkinson's disease.

4.2 Parkin disease: a phenotypic study of a large case series.

4.2.1 Introduction

This report provides a detailed clinical evaluation of twenty-four patients with parkin disease identified by the Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London.

4.2.2 Methods

4.2.2.1 Blood Collection and Genomic DNA extraction was performed as previously described.

4.2.2.2 Molecular Analysis

PARK2 PCR amplification, semiquantitaive PCR and sequence analysis was performed by Elizabeth Graham, Laboratory Technician, Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London; 2 coding exons of the parkin gene were amplified from genomic DNA by the polymerase chain reaction (PCR) using primers previously described (Kitada *et* al.1998) except for the primer for exon 3, for which exonic primers Ex3iFor and Ex3iRev were used (Abbas *et al.*, *1999*). The same primers were used for the sequencing of the PCR products of the 12 exons on both strands using Big Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing kit (Applied Biosystems, Foster City, CA), on an ABI 373 automated sequencer with the Sequence Analysis v.3.4.1 (Applied Biosytems) software. The methodology did not screen for rearrangements such as duplications and triplications.

4.2.2.3 Patients

115 DNA samples from patients with an age of onset of parkinsonism <50 years or unusual features were selected from the Research Laboratory, Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London. Patients had been referred directly from the National Hospital for Neurology, Queen Square, London (Professors AJ Lees, N Quinn and K Bhatia). Twenty-four were patients with parkin mutations (23 of whom had disease onset at or below age 35 years) studied using a retrospective review of medical records incorporating a standardised clinical proforma including a review of prior drug exposure, family and clinical history and examination. Cognitive assessment was performed using the Folstein Mini-Mental State Examination (Folstein et al., 1975) at varying intervals after disease onset. All subjects gave informed consent and the project was approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, London, UK.

A total of five first and second degree relatives without parkinsonism of five unrelated parkin patients were also examined and screened for parkin mutations.

4.2.3 Results

4.2.3.1 Molecular Analysis

The mutations in four isolated cases and five sibling sets have previously been reported (Lucking et al., 2000); these cases were re-screened by Elizabeth Graham (Khan et al., 2003) to ensure mutations found were correct. In addition eight new cases (seven isolated cases, one of whom was the product of a consanguineous marriage, and one additional member of one of the affected sibships) were identified by Elizabeth Graham (Table 4.2.3.1) (Khan et al., 2003). Mutations on both alleles were identified in fourteen cases, of which only one was a homozygote (patient 20), the remaining thirteen cases being compound heterozygotes carrying a different parkin mutation on each allele (three isolated, ten familial cases). In the remaining ten parkin cases (seven isolated, three familial cases) a single mutant allele was identified. As a group, mutations occurred most frequently in Exon7. Patients10 and 21 and siblings 15 and 16, shared point mutation Ex7 924C→T (Arg275Trp). Patients 10 and 17, and siblings 15 and 16 shared point mutation Ex12 1390G→A (Gly430 Asp). Patient 6 and his sister, patient 7 shared point mutation Ex9 1101C→T (Arg334Cys) with patient 14. Patients 11 and 20 shared exon 2 202-203 AG deletion. Patients 8 and 19 both had a deletion in exon 5. Patients 11 and 21-24 shared Ex9 1101C→T (Arg334Cys).

4.2.3.2 Patients

In all cases, the pattern of inheritance was compatible with autosomal recessive disease (clinically unaffected parents). Eleven patients were isolated cases; ten of them have young onset parkinsonism, but in the eleventh, the only one in the entire series from consanguineous parents, age at onset was 54 years. The remaining thirteen patients were from five unrelated families (Table 4.2.3.1). In one of these

Table 4.2.3.1. Parkin mutations identified in twenty four cases with parkinsonism

Patient	Isolated / Familial case		Parkin mutation Allele 1		Parkin mutation Allele 2
1	isolated		exon 6/7 deletion		uncharacterised
2	familial]		exon 8 deletion		intron 5 (+2 T>A)
3	familial]	sibs	exon 8 deletion		intron 5 (+2 T>A)
4	familial]		exon 8 deletion		intron 5 (+2 T>A)
5	familial]		exon 8 deletion		intron 5 (+2 T>A)
6	familial }	sibs	exon 9 1101C→T	(Arg334Cys)	exon 7 939G →A
7	familial }		exon 9 1101C→T	(Arg334Cys)	exon 7 939G →A
8	isolated		exon 5 deletion		uncharacterised
9	isolated		exon 12 1390G →A	(Gly430 Asp)	uncharacterised
10	isolated		exon 7 924C →T	(Arg275Trp)	uncharacterised
11	isolated		exon 7 905T →A	(Cys268 stop)	exon 2 202-203 AG deletion
12	familial }	sibs	exon 3 deletion		exon 4 deletion
13	familial }		exon 3 deletion		exon 4 deletion
14	isolated	(consanguineous)	exon 9 1101C →T	(Arg334Cys)	uncharacterised
15	familial }	sibs	exon 7 924C →T	(Arg275Trp)	exon12 1390G →A
16	familial }		exon 7 924C →T	(Arg275Trp)	exon12 1390G →A
	isolated		exon12 1390G →A	(Gly430As)	exon 3 deletion
18	isolated		exon 7 867C →T	(Arg256Cys)	uncharacterised
19	isolated		exon 5 deletion		uncharacterised
20	isolated		exon 2 202-203 AG deletion		exon 2 202-203 AG deletion
21	isolated		exon 7 924C →T	(Arg275Trp)	exon 5/6 deletion
22	familial }	sibs }	exon 7 905T →A	(Cys268Stop)	uncharacterised
23	familial }	}	exon 7 905T →A	(Cys268Stop)	uncharacterised
24	familial	uncle }	exon 7 905T →A	(Cys268Stop)	Uncharacterised

} = familial

Table 4.2.3.2.a Clinical characteristics of individual parkin cases: AO = age of onset parkinsonism DD = disease duration

Patient	**Sex/Age**	**Ethnicity** **Family History**	**AO**	**DD**	**H&Y** **on**	**off**	**Additional Features**	
1	F/ 34	Japanese	23	11	-	-	dramatic response to benzhexol, L-Dopa sensitivity ++	
2	F/54	Irish}	31	23	3	4	abduction-adduction oscillatory tremor of lower limbs)	FH suicide,
3	M/60	Irish}	28	32	2.5	4	abduction-adduction oscillatory tremor of lower limbs)	psychosis,
4	M/55	Irish}	26	29	3	4	abduction-adduction oscillatory tremor of lower limbs)	depression,
5	M/51	Irish}	32	19	3	4	abduction-adduction oscillatory tremor of lower limbs)	tremor
6	M/55	Indian}	28	27	2.5	3	misdiagnosed as DRD at onset	
7	F/46	Indian}	30	16	-	-	R sided hemi-parkinsonism at onset, bilateral STN DBS	
8	M/40	English	29	11	1	2	R leg tremor at onset, still L-Dopa naive	FH tremor
9	M/32	English	7	25	3	4	cervical dystonia at onset, misdiagnosed as DRD, sensitivity L-Dopa ++ atremulous, panic attacks & self mutilation	
10	M/30	Scottish	18	12	0	2.5	paranoia & panic attacks prior to parkinsonism, exercise-induced dystonia at onset	FH anorexia nervosa + depression
11	F/ 33	English	19	14	1.5	2.5	bilateral akinetic rigid, dramatic response benzhexol, FH depression still L-Dopa naïve	
12	M/77	Dutch }	30	47	2	3	early falls, atremulous, diagnosed and treated after 20 years, dramatic response to L-Dopa within 2 hours	FH suicide
13	M/74	Dutch }	20's	>45	3	4	tremor at onset, dramatic response to L-Dopa	
14	M/58	Bangladeshi	54	4	1	2	consanguineous parents, prominent orofacial dyskinesias 3 days after starting L-Dopa.	
15	M/51	Irish }	13	38	3	4	DRD considered at onset, psychosis, L-Dopa sensitivity ++	
16	F/56	Irish }	34	22	2	2.5	postnatal depression prior to onset, L-Dopa sensitivity ++	
17	M/29	Irish	13	16	2.5	4	dramatic response to benzhexol, autonomic & axonal FH depression peripheral neuropathy, still L-Dopa naïve, attempted suicide	
18	F/21	English	13	8	-	-	coincidental cerebral palsy	
19	M/31	English	10-15	16-21	1	2	coincidental familial motor tics, neck tremor at 12-15, FH tremor	
20	M/57	English	29	28	1	3	drug-induced psychosis, unilateral thalamotomy FH depression	
21	M/52	English	22	30	1	4	writer's cramp at onset, L-Dopa sensitivity ++, FH depression	
22	F/36	English} Burnley	15	21	-	-	anorexia nervosa, suicide attempts, depression prior to onset, bilateral thalamotomy	
23	F/46	English}	15	31	3	4	good response to benzhexol, still L-Dopa naive	FH tremor
24	M/80	English} kindred	24	56	-	-	dramatic response to L-Dopa, +depression	

} = familial - = unavailable motor scores families, the Burnley kindred (patients 22-24), young onset parkinsonism was observed in two generations (Figure 4.2.3.2). All cases, except patients 17 and 18, fulfilled the UK Queen Square Brain Bank diagnostic criteria for idiopathic Parkinson's disease except for the presence of a family history (Gibb et al., 1988). Sixteen cases were male and eight female (Table 4.2.3.2.a). All cases were resident in the UK but were of differing ethnic origins: Irish (isolated case n=1, families n=2), English (isolated cases n=7, family n=1), Scottish (isolated case n=1), Japanese (isolated case n=1), Indian (family n=1), Bangladeshi (isolated case n=1), Dutch (family n=1). The age at onset of symptoms in all twenty four cases ranged from 7 to 54 (mean 24.0 +/- 9.9SD) years. Disease duration ranged from 4 to 56 (mean 24.2 +/- 13.0 SD) years (Table 4.2.3.2.b).

4.2.3.2.1 Motor features

At disease onset, bilateral symptoms were reported in 26%, limb tremor in 70%, bradykinesia in 44% and micrographia in 13%. The overall picture was that of slowly progressive parkinsonism thus despite frequently long disease duration, none of the patients was greater than Hoehn & Yahr stage 3 in 'on' or stage 4 in 'off' at the last evaluation, and patient 12, despite disease duration of 47 years, was still stage 2 in 'on' and stage 3 in 'off' (Table 4.2.3.2a). 23% of cases reported "poor balance" as an initial symptom. 63% of cases reported freezing, which developed in 8% of them within 5 years, and in 33% within 10 years of disease onset. 50% of cases reported 1-6 falls per month, 30% of them within 5 years and 50% within 10 years of disease onset. 63% reported sleep benefit.

Table 4.2.3.2b Group clinical characteristics of patients with parkin disease.

age of onset	**number of cases**
<20	9
21-30	11
31-40	3
41-50	0
51-60	1

Mean age of onset all cases in yrs (SD, range)	24.0	(+/- 9.9, 7-54)
Mean disease duration in yrs (SD, range)	24.5	(+/-13.1, 4-56)

Symptoms and signs		
at onset	tremor	70%
	bradykinesia	44%
	rigidity	13%
	dystonia	41%
	symmetry	26%
	micrographia	13%
	poor balance	23%
at examination		
	bradykinesia	100%
	rigidity	100%
	rest or postural tremor	92%
	brisk reflexes	30%
	Mini Mental Test Score	28 (range 25-30)
Autonomic symptoms		60%
	urgency	45%
	impotence	28% of males
	hypotension	13%
Freezing episodes		63% (8% within first 5 yrs)
Falls		50% (30% within first 5 yrs)

Response to L-dopa / anticholinergics	
excellent	75%
psychiatric	56%
duration of L-dopa treatment (n= 16/24 patients)	15.9 (9.0SD)
dyskinesias in L-dopa treated patients	100%
fluctuations in L-dopa treated patients	50%

Examples:

Patient 1. This Japanese woman developed a limp of her right leg at age 23. A year later her right ankle would invert at rest on the bed, but not on walking, and at age 26 she complained of tremor, balance difficulty and retropulsion and was diagnosed with Parkinson's disease. She was treated with benzhexol 1mg three times a day and was subjectively 100% better. At age 28 because of nausea on benzhexol she was changed to taking Madopar (L-Dopa plus benserazide), and at age 34 she was taking 1 tablet of Madopar dispersible 62.5 (12.5/50) in the morning followed by half a tablet (L-Dopa content 25mgs) every hour or so thereafter. Higher individual doses caused unacceptable dyskinesias. At age 29 she was treated for symptoms of depression.

4.2.3.2.2 Tremor

With the exception of patients 10 and 12, who still remained atremulous after disease durations of 25 and 47 years respectively, the remaining 22 (92%) developed a tremor (at rest or postural) at some stage of disease. Examples:

Patients 2-5. These four siblings, from an Irish kindred, shared in common a marked resting leg tremor with abduction-adduction oscillations (Khan et al., 2002).

Patient 6. This 55 year old man developed a tremor of the right big toe at age 28 years which subsequently involved both legs, more on the right, and was present at rest, supine and standing. He reported no diurnal fluctuations. Following a marked response to L-dopa he was misdiagnosed as tremor-dominant DRD. Six years after starting L-Dopa therapy he developed dose-dependent peak dose dyskinesias and wearing off and his diagnosis was revised.

Patient 7. This 46 year old sibling of patient 6, developed at age 30 years right-sided hemi parkinsonism with dystonia of the right arm and foot, without diurnal fluctuation, which progressed to a bilateral akinetic-rigid picture with a coarse rest tremor confined to the legs. At age 35 years she had subthalamic deep brain stimulators implanted bilaterally with good effect.

Patient 8. This man developed tremor of the left leg at age 29 years, which subsequently involved the right leg and right hand, and was diagnosed to have essential tremor. At age 34 years he noticed slowness of movement, having to check his balance, and diminution of arm swing on the right. His left toes would claw when sitting or driving. He also reported panic attacks. At age 40 years he commenced orphenadrine with resolution of all symptoms except tremor: he remains L-dopa naive. Both his father and his 42 year old sibling, (genotype uncharacterised) report an isolated tremor of the arms

4.2.3.2.3 Dystonia

Dystonia was reported in 41 % of cases as a presenting symptom, involving feet in seven, hands in two, and neck and trunk in one each. 78% had developed dystonia at some point prior to treatment, involving hands in three, feet in 18, neck in five, and trunk and gait in one each. Some had involvement of more than one of the above sites. Examples:

Patient 9. This patient developed stiffness of his trunk and legs, turning and stiffness of his neck, poor balance and an inability to stand upright without toppling backwards at age 7 years. He progressed to an atremulous akinetic rigid syndrome with cervical dystonia and dystonia of both feet. He showed a dramatic response to L-Dopa and was initially suspected to have DRD. However, he developed wearing off and had markedly diminished striatal ^{18}F-dopa uptake on PET scan (Sawle, et al, 1991, case 7), confirming juvenile parkinsonism. At age 29 he developed laryngeal adductor dystonia requiring botulinum toxin injections. Otherwise when 'on' he was physically almost normal. He could not tolerate more than 50 mgs of L-Dopa with a peripheral dopa decarboxylase inhibitor (PDI) at each of his eighteen daily doses because this worsened his laryngeal dystonia. At age 31 he was complaining that during off periods he had "no sense of balance" and had some freezing, festination and retropulsion, leading to falls. At age 22 he developed panic attacks and at age 32 had attempted self mutilation in the form of forearm and wrist slashing. He has recently been started, aged 32, on an apomorphine pump.

Patient 10. This 30 year old man developed inturning of the right foot at age 18 years whilst playing football. At that time he also reported occasional paranoia and panic attacks. At age 21years, he developed a tremor of the right thumb, and by age 26 years had developed rigidity and bradykinesia that improved subjectively by 50% on amantadine. He was treated for depression at the age of 29 years. One of his parents, who carries a mutant parkin allele, had been treated for depression for over 20 years. His 27 year old sister, (genotype uncharacterised) who does not have parkinsonism, has a five year history of anorexia nervosa.

4.2.3.2.4 Response to treatment.

An excellent and sustained response to drug therapy was reported by 75% of treated patients.

Dramatic response to anticholinergics:- Patient 11 This patient reported poor balance, clumsiness, and difficulty rising from a seated position beginning at age 19 years. By age 25 she had developed a shuffling, small-stepped gait, especially in confined spaces. Examination revealed a bilateral akinetic rigid syndrome with a very fine postural tremor in all four limbs and brisk reflexes. She also reported severe mood swings and exacerbation of her motor disability by alcohol. Treatment was started with benzhexol and after two days she said she was "100% better". She reported marked pre-menstrual worsening of motor disability. She is still, aged 33, well controlled on benzhexol 2mgs three times a day, and has not required dopaminergic therapy. She reports severe mood swings during her teenage years and also a maternal aunt (genotype uncharacterised) with depression.

Dramatic response to levodopa:- Patient 12. This 77year old man is one of ten siblings of whom another brother, patient 13, and a sister (who is not reported in this series) have parkin disease. He noted slowness of day to day tasks and frequent falls from 30 years of age. At age 50 he developed dystonia of both feet and micrographia and was diagnosed with parkinsonism, 20 years after his first symptoms. He reported a dramatic response within two hours of taking his first dose of 150 mg of L-Dopa (plus PDI). He remains free of tremor after 47 years of disease, and is currently taking Sinemet (L-Dopa plus carbidopa) 25/100 four times a day.

Patient 13. This 74year old brother of patient 12, reported dragging his left foot, a lazy left arm, and tremor of all four limbs in his late twenties. He also reported a dramatic improvement of symptoms when he started L-Dopa treatment at age 68 years. His cognitive function remains normal after more than 45 years of disease. He reports a brother (genotype uncharacterised), who did not have parkinsonism who committed suicide at age 43.

4.2.3.2.5 Treatment related complications

Sixteen patients had started L-Dopa therapy, with a mean treatment duration of 15.9 +/- 9.0SD years. 50% of them reported dose-related fluctuations, and all reported peak dose dyskinesias, except one patient who reported diphasic dyskinesias. Dyskinesias had developed after a mean interval of 6.7 years (+/- 7.6SD) (range from 3 days to 21 years) after commencing L-Dopa treatment. Some patients developed atypical or unusual levodopa-induced dyskinesias, or excessive sensitivity to very low doses of L-Dopa.

Patient 14. This 58year old man, whose parents were first cousins, developed asymmetrical parkinsonism with dystonic posturing of the trunk at age 54 years. Within 3 days of commencing L-Dopa therapy he developed prominent orofacial dyskinesias and mild dyskinesias of the lower limbs.

Patient 15. This 51 year old man developed a fine tremor of his right limbs aged 13 and at age 22 dystonia of his right foot, which dragged after prolonged walking. At age 26 he started Sinemet for his dystonia-parkinsonism with considerable benefit, but with wearing off from the very beginning of treatment. Although there was no diurnal variation prior to treatment he was initially considered to have DRD. On L-Dopa, he developed a very bizarre scissoring dystonic gait. When overdosed this gait was even worse, and was accompanied by neck bobbing, generalised choreiform and myoclonic movements, and repetitive tongue protrusion. His dosage threshold for developing this gait has become extremely low. At age 47 he found that after one tablet of half Sinemet CR 25/100 (containing 70 mgs biovailable L-Dopa) he was overdosed, but after a half tablet he was good initially but then overdosed after an hour, and he settled on taking a quarter to a third of a Sinemet CR 50/200 three to five times a day. In addition to the sensitivity to dyskinesias, he has twice developed hypomania. On the first occasion aged 36 years he was taking Sinemet 10/100 six times day and no other medication, and on the second occasion aged 46 he was on Sinemet 25/100 six tablets a day together with Madopar dispersible 25/100 six tablets a day. Patient 16, his 56year old sister, developed parkinsonism at age 22 years after being treated for postnatal depression with chlorpromazine. She reports that she develops marked depressive symptoms if she takes more than 50mg of L-Dopa at any time.

4.2.3.2.6 Autonomic dysfunction.

Autonomic symptoms were present in 60% of patients, with 45% reporting urinary urgency, 28% of male patients reporting impotence, and 13% reporting orthostatic symptoms. Example:

Patient 17. This 29year old man developed parkinsonism at the age of 13 years. At age 16 he reported dizzy spells without loss of consciousness, related to orthostatism and exercise. At age 20 it was noted that he did not perspire and he reported loose stools and intermittent difficulty with erections and ejaculation. At age 24 he developed urgency of micturition. Autonomic function tests confirmed both sympathetic and parasympathetic cardiovascular autonomic impairment. Reflexes were brisk and sensation was normal but nerve conduction studies and nerve biopsy revealed an axonal peripheral neuropathy. After an initial dramatic response to low doses of benzhexol, he continues to show a moderate motor response after seven years taking benzhexol alone and seventeen years of disease. At age 18 he attempted suicide by taking an overdose of painkillers and age 21 was treated for depression. His unaffected sibling, who carries a single mutant parkin allele, has been treated for depression.

4.2.3.2.7 Other clinical findings

Cognition was normal in all cases, with a mean mini mental test score of 28, except for patient 18 (see appendix), who had presumed cerebral palsy. Hyperreflexia was present in 30% of cases. Eye movements, sensory testing and co-ordination were normal in all subjects except for patient 18. Patient 18 with presumed cerebral palsy, and patient 19 with a past and family history of tics, had other neurological abnormalities that we believe are probably, but not necessarily, coincidental.

Patient 18. This 21year old woman, with presumed cerebral palsy secondary after peri-partum anoxia, developed a spastic diplegia at age 12, and one year later developed left sided parkinsonism presenting with a rest tremor of the left foot and dystonic posturing of both feet. There was some improvement with a trial of L-Dopa. At age 19 years she developed off periods and a year later started to have grand mal seizures. She also had head banging, biting and scratching and severe cognitive, visual and hearing impairment.

Patient 19. This 32year old man had developed an intermittent neck-shuddering tic and a no-no head tremor between the ages of 10 and 15 years, and nose wrinkling and jaw deviation tics at age 29 years. There were no vocalisations, obsessions or other features of Tourette syndrome. A maternal uncle was reported to have a facial twitch. At the age of 30 years he developed right-sided parkinsonism, without diurnal variation, at which time his tics were noted to have worsened. His mother, who carries a mutant parkin allele, and his sister (genotype uncharacterised), have been treated for depression. A 24 year old maternal first cousin (genotype uncharacterised), had been diagnosed with essential tremor at the age of 14 years.

4.2.3.2.8 Behavioural Disorder

56% of cases reported psychiatric/behavioural symptoms (Table 4.2.3.2.8). In 25% these symptoms started prior to, and in 31% after, the onset of parkinsonism.

Patient 20. This 57year old man reported tremor of the left foot and stiffness and aching of the left leg from age 29 years which progressed to left hemi-parkinsonism. He was treated with Sinemet with a very good result, but developed motor fluctuations and dyskinesias. At age 37 years he underwent right-sided thalamotomy with complete resolution of his left-sided tremor for 5 years. At age 46 years, taking L-Dopa, he developed some features of hypomania, particularly when in on periods.

At age 47 years an apomorphine pump was added with benefit. The following year, the day after adding selegiline to his regime, he became psychotic, with paranoid delusions and third person auditory hallucinations, which lasted three months until apomorphine was stopped. Later apomorphine was restarted. At age 49 he was admitted because of painful off periods and on period hypomanic symptoms, and again became psychotic. This resolved after again stopping apomorphine and he was abulic and possibly depressed for the next 5 years, but then spontaneously emerged from this state. When he was reviewed at age 58 he had no further psychiatric problems other than feeling a bit high in his on periods, despite being on a cocktail of Sinemet (1130 mgs L-Dopa, plus PDI, per day), amantadine, pramipexole, and low doses of pergolide, ropinirole and entacapone. He was on, with mild to moderate dyskinesias but no functional impairment, for almost all of the time, but when off was unable to walk. His father (genotype uncharacterised) had a history of mood swings, depression and personality change from age 58 years and died at age 79.

Patient 21. This 52year old man reported difficulty with writing at age 22 years and two years later was diagnosed with writer's cramp. At age 26 years he reported occasional curling of the toes of the left foot solely with exercise, and was treated with orphenadrine with considerable benefit. Over succeeding years he developed increasing difficulty walking with start hesitation, a tendency to freezing, “tottering” and occasional shuffling, but no falls. On examination he was noted to have asymmetric parkinsonism with a right “striatal toe”. At age 42 he was started on an L-Dopa preparation with striking benefit, with the addition of lisuride shortly thereafter and then selegiline. Within 18 months he had developed some motor fluctuation and his main problem was start hesitation in the evenings. He had also

Table 4.2.3.2.8. Parkin cases with behavioural disorder and a family history of behavioural disorder.

Prior to onset of motor symptoms

Depression	2	(patients 11, 16)
Paranoia	1	(patient 10)
Psychosis	0	
Panic attacks	1	(patient 10)
Anorexia nervosa	1	(patient 22)
Suicide attempts	0	
Self harm	1	(patient 18)

After the onset of motor symptoms

Depression	5	(patients 1, 10, 15, 17, 20)
Paranoia	0	
Psychosis	3	(patients 15, 20, 21)
Panic attacks	5	(patients 2, 4, 9, 10, 21)
Anorexia nervosa	0	
Suicide attempts	2	(patients 17, 21)
Self harm	2	(patients 8, 9)

Behavioural disorder in first and second degree relatives of parkin patients

Depression	8	(patients 2-5, 10, 11, 17, 19-24)
Paranoia	0	
Psychosis	2	(patients 2-5, 21)
Panic attacks	0	
Anorexia nervosa	1	(patient 10)
Suicide attempts	2	(patients 2-5, 12-13)
Self harm	0	

developed some features of hypomania. Increasingly he developed on period freezing that was absent in the morning before his first dose, and worse the higher the dose of L-Dopa he took, so his optimal regime was just over half a tablet of Sinemet 12.5/50 (containing 25mgs L-Dopa) at each intake. His greatest concern was a combination of start hesitation, freezing and retropulsion. At age 49 he developed delusional morbid jealousy and his lisuride was stopped. Subsequently he stopped Sinemet and was treated with agonists alone with good motor control but again developed morbid jealousy on cabergoline and later on ropinirole. He attempted suicide on one occasion and also developed panic attacks. He reports a 64year old maternal first cousin (genotype uncharacterised) who has a paranoid psychotic illness.

4.2.3.2.9 Family History

Motor features

Five relatives of index cases (patients 2, 8, 19, 22) were reported to have isolated tremor, essential tremor or postural tremor. Subtle extrapyramidal signs have been reported in three asymptomatic parkin carriers of the Irish kindred (patients 2-5) (section 5.2.3.1).

Behavioural features

The sixteen unrelated parkin patients reported a total of twelve of 71 (17%) first or second degree relatives without symptoms of parkinsonism, but with a history of psychiatric illness (Tables 4.2.3.2.a and 4.2.3.2.8). Five of these relatives were screened for parkin mutations: all five were carriers of a single mutation and all had a normal neurological examination except for a sibling of patient 2, who had subtle extrapyramidal signs that did not fulfil diagnostic criteria for IPD. However, this relative had a history of depression and schizophrenia, and had been treated with neuroleptics (Khan et al 2002).

The Burnley kindred (see Figure 4.2.3.2).

This kindred originated from Burnley, a small town in Lancashire, England. Grandparents I.1, I.2 and parents II.1, II.2 were from the same town, but were reported not to be related and did not have parkinsonism. Patients 22 (III.2), 23 (III.4) and 24 (II.7) were members of this family. This family is unusual in that parkinsonism occurred in two generations, with all affected individuals sharing the same parkin mutation on one chromosome, with the other allele being undetermined.

Patient 22 (III.2, Figure 4.2.3.2), a non-identical twin, was diagnosed with anorexia nervosa at age 14 years. At that time she weighed three and a half stone, became socially reclusive, attempted suicide on several occasions and was treated with chlorpromazine and orphenadrine. From age 15 she developed parkinsonism, presenting with an asymmetrical (L>R) tremor of all four limbs that occurred both at rest and with movement. At age 27 years she had a right-sided thalamotomy without benefit, and at age 28 years underwent left-sided thalamotomy with clinical improvement. At 37, following a suicide attempt, she died of bronchopneumonia. The coroner's autopsy did not provide useful information about the brain.

Patient 23 (III.4, Figure 4.2.3.2), a 46 year old housewife, developed a tremor of the right hand at the age of 15. At age 17 years she noted inturning of both feet on walking and progressed to right sided hemi-parkinsonism.

Patient 24 (II.7, Figure 4.2.3.2) developed parkinsonism from age 25 years and died aged 80 years. This individual had four children, one of whom, III.10 (genotype uncharacterised) has a postural tremor at age 32 years.

Individual II.6 (genotype unknown, Figure 4.2.3.2) was reported by relatives to have developed a unilateral coarse rest tremor at the age of 65 years. This progressed to Levodopa-responsive parkinsonism and the patient died aged 77 years. II.6 had four children (genotype uncharacterised), two of whom have been treated for depression. II.3 and II.4 (genotype uncharacterised, Figure 1) were examined aged 70 and 82 years respectively: a postural tremor was noted with no signs of parkinsonism.

4.2.4 Conclusion

Parkinsonism due to mutations in PARK2 also referred to as parkin disease, is a distinct genetic entity with a phenotype that is typically characterised by young onset (<40 years) parkinsonism, predominant lower limb dystonia, dramatic response to L-Dopa and a benign and slowly evolving course (Khan et al., 2002). Whilst young age of onset is undoubtedly the best clinical indicator of parkin disease, the condition is not confined to young or juvenile cases: one familial case had an age of onset of 54 years. In the Burnley kindred, II.6 (genotype unknown) Figure 4.2.3.3, who is deceased, developed parkinsonism aged 65 years. This individual may also have had parkin disease although IPD could not be excluded. These cases extend previous reports of some familial cases having an age of disease onset closer to the average for IPD (Lucking et al., 2000, Klein et al., 2000). The phenotype can also include a bilateral atremulous, akinetic-rigid syndrome, and unusual tremor such as an abduction-adduction tremor confined to the legs in patients 2-5 or the tremor-dominant parkinsonism reported in an Italian kindred (Hilker et al., 2001). The clinical presentation of most cases was broadly comparable to that reported in ARJP and YOPD in addition to the IPD (Klein et al., 2000) and DRD phenotypes (Tassin et al., 2000) that have previously been reported. (Table4.2.4). Due to the difficulty in differentiating between DRD and a DRD-like presentation of parkin disease and other cases of juvenile parkinsonism, exclusion of parkin mutations or the use of DaT SPECT or ^{18}F-Dopa PET scans may be indicated in DYT-1 negative patients with young-onset dystonia-parkinsonism, in order to delay the complications of L-Dopa therapy in young onset parkinsonism.

Brisk reflexes may be present in parkin disease and two previous reports have confirmed cerebellar and pyramidal tract dysfunction in parkin subjects (Kuroda et al., 2001, van de Warrenburg et al., 2001); however in this series, cerebellar and pyramidal signs were absent, apart from brisk reflexes in case 11. Additionally two cases were accompanied by presumed coincidental cerebral palsy in one and a familial tic disorder in another.

Three new presentations of the disease are noted. Patient 17 developed early autonomic dysfunction combined with an axonal peripheral neuropathy. The presence of axonal peripheral neuropathy alone has previously been reported in a single parkin case (Tassin et al., 1998). Autonomic symptoms alone are common (Yamamura et al., 2000), and were present in 60% of our patients. Patient 10 presented with cervical, and later developed laryngeal, dystonia. Patient 9 developed pure dystonia of the foot on exercise.

There was also a patient with writer's cramp at onset, a phenotype which has previously been reported (Farrer et al., 2001).

Cognitive function remains normal in the majority of patients (Lucking et al., 2000). In this series all subjects, except patient 18, with presumed cerebral palsy, had normal cognition, or at least normal scores on the MMSE. Indeed normal cognition was recorded in patient 13 after over 45 years of parkin disease, implying that the neurodegeneration probably spares structures critical to cognitive function. Several of the patients studied developed repeated psychosis, one of whom was taking L-Dopa

Table 4.2.4. Key features of parkin disease (including our cases)

- age of onset usually ≤40 years
- typical presenting phenotype YOPD/ ARJP
- normal cognition
- frequent foot dystonia
- early instability, freezing, festination or retropulsion in some cases
- excellent response to anticholinergics in some cases
- excellent response to L-Dopa, but frequent development of exquisite sensitivity to low doses.
- atypical levodopa-induced dyskinesias in some cases
- behavioural disorder preceding onset parkinsonism and complicating treatment in some cases
- autonomic symptoms in many cases
- usually benign and slow clinical course
- atypical presenting phenotypes include:

    later onset, mimicking IPD
    exercise-induced dystonia, DRD-like
    leg tremor
    atremulous bilateral akinetic rigid syndrome
    focal dystonia (writer's cramp, cervical)
    autonomic or peripheral neuropathy
    cerebellar and pyramidal tract dysfunction

- coincidental neurological disease does not exclude the diagnosis
- family history of individuals with tremor, subtle extrapyramidal signs and behavioural disorder needs further investigation

preparations alone. In the four parkin cases from elsewhere whose autopsy results have been published the pathology, as in IPD, has involved the substantia nigra and locus coeruleus, but other brain structures that are involved in IPD are usually spared. Although it is conceivable that the greater degree of nigrostriatal denervation in caudate nucleus in parkin disease might play a role in the behavioural disturbances observed in a number of our patients, it is possible that they may have other pathological or neurochemical abnormalities that remain to be identified. Behavioural disorders in parkin disease have previously been reported (Tassin et al, 1998, Yamamura et al, 2000), but not in detail. Moreover, psychiatric symptoms antedating the onset of parkinsonism have not hitherto been described or emphasised. This may represent another new presentation of parkin disease. However, this would require confirmation by studying a larger series of cases and controls. Psychiatric complications, including depression, anxiety and psychosis have been reported in idiopathic PD, with rates for depression ranging from 20-50% (Oertel et al, 2001, Schrag et al, 2001), anxiety disorders from 20-40% (Aarsland et al, 1999) and psychosis from 15-20% (Aarsland et al, 1999). However, behavioural disturbances including anorexia nervosa, self-harm and suicide attempts are rare in PD but affected 24% of patients in this series.

A dramatic response to treatment with L-Dopa preparations is characteristic of both YOPD and parkin disease. It is intriguing that there is also a dramatic response to treatment with anticholinergics alone in both early and advanced stages of disease; this has been previously reported in a single parkin case (Munoz et al., 2000). Although anticholinergics may have a modest effect on tremor in IPD, they do not usually improve rigidity or akinesia. In contrast, patients 1, 11 and 17, who reported initial virtually complete resolution of symptoms on low doses of benzhexol, had no or minimal tremor. A dramatic benefit from anticholinergics is also recognised in patients with DRD (Jarman et al, 1997), in which there is also an impairment of central endogenous dopamine production in young subjects.

The dramatic response to L-Dopa usually seen in parkin patients indicates that, despite a generally more severe striatal dopaminergic deficit in uptake and storage of L-Dopa and its metabolites as revealed by ^{18}F-Dopa PET, administration of even low dosages of L-Dopa is nonetheless able to restore many subjects motorically almost to normal. The exquisite sensitivity of parkin patients to low doses of L-Dopa may partly reflect post-synaptic striatal dopamine receptor supersensitivity secondary to their severe presynaptic lesion.

L-Dopa induced dyskinesias are common in parkin cases. In our patients, the average interval from initiating L-Dopa to developing dyskinesias was 6.7 years, which is longer than the average in our two reported series of patients with YOPD (Quinn et al, 1987, Schrag et al, 1998). L-Dopa-induced dyskinesias in some of the patients were unusual, or extremely dose-sensitive. For example, patient 14 developed prominent orofacial and mild limb dyskinesias only two days after commencing L-Dopa, and patient 15 developed a peak-dose dystonic scissoring gait after only very small amounts of L-Dopa. Interestingly, only one of our cohort of 16 L-Dopa treated parkin patients reported diphasic dyskinesias compared to 15 of 45 cases of YOPD treated with L-Dopa for 2 years or more (Quinn et al., 1987).

Freezing, festination, retropulsion, instability and falls are usually considered late features in patients with IPD, and are often assumed to be extra-nigral or non-dopaminergic in origin. However, these can be early or presenting features in some parkin patients. This might be due to a more severe nigral lesion affecting the caudate more than in IPD, or alternatively to additional, as yet unrecognised, pathology.

Parkin disease is typically autosomal recessive and seen in one generation, among male and female siblings who are equally affected. However, it can also appear in isolated cases. In addition it has been reported in multiple generations of families with consanguineous marriages (Maruyama et al., 2000) or in isolated populations (Klein et al., 2000, Lucking et al., 2001). The latter, and the finding of parkin disease in two generations in the Burnley kindred, suggests that different parkin mutations are frequent enough in some populations to lead to allelic heterogeneity. However, the frequency of parkin carriers and the frequency of the disease (both typical and atypical phenotypes) in the population at large remains to be accurately determined.

The majority of parkin mutations have arisen from independent mutational events, emphasising their importance in the aetiology of young onset parkinsonism (Abbas et al., 1999, Lucking et al. 2000). However, recurrence of the same mutation in different patients may reflect a founder effect (i.e. these individuals have a common ancestor and are therefore related), which is particularly the case with respect to point mutations (Periquet et al., 2001). Different patients from apparently different families shared the same mutations: three patients from two families originating from the Indian subcontinent shared exon 9 1101C→T (Arg334Cys) three patients from two families of Irish descent and one Scottish isolated case shared exon 12 1390G→A (Gly430 Asp) and two isolated English cases shared an exon 2 202-203 AG deletion. One isolated English case and all siblings from the Burnley kindred shared Ex9 1101C→T (Arg334Cys). Four cases (two Irish from one family and two isolated English cases) shared exon 7 924 C →T (Arg275Trp), which has been reported to be due to very ancient founder effects (Periquet et al., 2001). The majority of mutations in our patients were due to different point mutations, in contrast to the deletions first reported in the Japanese parkin patients (Kitada et al., 1998). A second parkin mutation was not identified in ten of the parkin patients. Possible explanations for this could include: an incomplete mutation screen (incomplete analysis of the parkin promotor and intronic regions); the presence of an unknown coexisting susceptibility allele; or that a single mutant allele is sufficient to cause disease.

With recent genetic advances, we now realise that the clinical heterogeneity of "Parkinson's disease" is, at least in part, due to the fact that it encompasses a number of different disease entities. Autosomal recessive conditions such as parkin disease can be due to different "loss of function" mutations, but in such cases the clinical phenotype is usually similar (Strachan, 2000). Although common and overlapping clinical themes have been reported in this series of parkin patients, they are nonetheless also clinically heterogeneous. Since a number of different mutations in the parkin gene are responsible, this may be mutation dependent. Although both this and previous studies are too small to confidently identify mutation-specific phenotypes, it is of interest that in the large Irish kindred all four siblings, patients 2-5, sharing the same mutations, presented between the ages of 26 and 32 years, and that all four of them had in common an abduction-adduction oscillatory tremor of the legs. By contrast however, in another sibling set, patient 13 is atremulous after 47 years of disease and his sibling, patient 12, had tremor at the onset of disease.

The significance of a 17% rate of psychiatric disturbance reported in relatives by sixteen unrelated patients with parkin disease is uncertain because we do not have a control cohort. The general population incidence of psychiatric disturbance of 19 % (Singleton et al; 2000) was ascertained from a household survey and therefore may not be directly comparable. Nigrostriatal dysfunction detected using ^{18}F-dopa PET and the manifestation of subtle extrapyramidal signs that do not fulfil clinical diagnostic criteria for IPD, have been reported in carriers of a single parkin mutation

(Klein et al., 2001, section 5.3), so-called "manifesting heterozygotes". One could hypothesize that parkin carriers who do not have parkinsonism but carry a single parkin mutation might also have susceptibility to psychiatric illness. In this context, it is of interest that a locus for schizophrenia has recently been mapped to chromosome 6q25 adjacent to PARK2 (Lindholm et al, 2001). The manifestation of behavioural disorder in non-parkinsonian carriers of a single mutant allele may support the hypothesis that by either *haploinsufficiency* (Strachan 2000) or a *dominant negative effect* (Strachan 2000), having gene products up to 50% of the normal level is not sufficient for normal function. However, the progression to develop parkinsonism in such cases is unclear. The significance of the reported behavioural disorders in otherwise unaffected relatives either with only one demonstrated mutation, or whose genotype is uncharacterised remains to be determined.

4.3 Olfaction in parkin disease compared to early-onset parkinsonism without parkin mutations and idiopathic Parkinson's disease.

4.3.1 Introduction

Loss of olfaction occurs in different forms of parkinsonism: severe loss is seen in idiopathic Parkinson's disease (PD) (Hawkes et al., 2003), diffuse Lewy body disease (DLBD) (Liberini et al., 1999) and parkinsonism-dementia complex of Guam (Doty et al., 1991), intermediate loss has been documented in multiple system atrophy (MSA) (Wenning et al., 1993), while olfaction has been found to be normal in corticobasal degeneration (Wenning et al., 1995), progressive supranuclear palsy (Wenning et al., 1995) and parkinsonism caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Doty et al., 1992). The exact cellular and molecular mechanisms underlying olfactory dysfunction in these disorders are unclear. However the detection of neuronal loss with Lewy bodies in the olfactory pathway in PD (Daniels et al., 1992) suggests that, at least in this disease the mechanism is central and neurodegenerative rather than a local nasal mucosal or sinus problem.

At the time of this study there were no reports on olfactory function in parkin disease. The aims of this study were to assess olfactory function in a group of patients EOPD with parkin mutations and to compare olfaction to a group of EOPD patients in whom mutations in PARK2 had been excluded and to a group of patients with PD. This study was performed in collaboration with Dr Regina Katzenschlager, Institute of Neurology, Queen Square who provided idiopathic PD cases and controls and Hilary Watt, London School of Hygiene and Tropical Medicine, Department of Statistics, University College London who advised about methodology of statistical analysis.

4.3.2 Methods

4.3.2.1 Subjects

17 patients with EOPD (defined as disease onset < 50 years of age) with mutations in PARK2 (parkin positive) who had been previously been identified (Lucking et al 2000, Khan et al,. 2002) served as the primary study group. PARK2 mutations on both alleles were identified in 12 parkin positive patients of which only one was a homozygote the remaining 11 patients being compound heterozygotes carrying a different parkin mutation on each allele (Lucking et al 2000, Khan et al,. 2002). In the remaining 5 parkin positive patients, a single mutant parkin allele was identified the second mutation remains undefined (Lucking et al 2000, Khan et al,.2002). 11 patients with EOPD who did not have mutations in the coding region of PARK2 (parkin negative) (Lucking et al 2000), 18 patients with PD and 28 normal subjects served as comparison groups. The groups of parkin positive and parkin negative patients were closely matched for age, sex and smoking status with one normal

control. Both groups were also closely matched for sex and smoking status with a group of PD patients; precise age-matching was not possible for EOPD patients because any patients who had early onset parkinsonism and would therefore be suitable for precise matching had been tested for parkin mutations and therefore, by definition, fell into either the parkin positive or parkin negative groups.

Selection criteria for the PD cohort included age of onset >50 years, fulfilment of the Queen Square Brain Bank criteria (Gibb et al., 1988), no family history of parkinsonism and no parental consanguinity. This cohort was not screened for PARK2, parkin mutations. All subjects gave informed consent and the project was approved by the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery (NHNN) and Institute of Neurology, London, UK.

4.3.2.2 Olfactory tests.

The University of Pennsylvania Smell Identification Test (UPSIT) was used to test olfaction. The UPSIT has been validated as a reliable standardised test of odour identification sensitive to a wide range of olfactory deficits (Doty et al., 1984). The standardised test consists of forty microencapsulated odorants embedded in urea-formaldehyde polymer spheres fixed in a proprietary binder and positioned in strips, one on each page of the test booklets. Subjects choose one out of four possible odorant options (forced choice). The UPSIT scores range from 0 to 40 and higher UPSIT scores denotes better olfactory function. All subjects were tested either during a routine clinic visit or specifically attended for the purpose of this study.

4.3.2.3 Statistical analysis.

UPSIT scores between the three study groups were compared using non-parametric analysis (Mann Whitney U test). Linear regression was performed to adjust for the age difference between the PD group and all other groups. This was performed by

Hilary Watt, London School of Hygiene and Tropical Medicine, Department of Statistics, University College London.

4.3.3 Results

All EOPD patients fulfilled the Queen Square Brain Bank criteria for PD (Gibb et al., 1988). Table 4.3.3 shows demographic and clinical details of the four study groups. UPSIT test scores are shown in Table 4.3.3 and Figure 4.3.3. There were no significant differences in the UPSIT scores of the parkin positive group of EOPD patients compared to healthy controls (p=0.20). The mean UPSIT score in the parkin positive group was 27.3 (95% confidence interval CI 24.4, 30.2). This did not differ significantly from the normal control group mean of 29.4 (95% CI 28.0, 30.7; p=0.20). In the parkin positive group individual UPSIT scores were above 23 or more except for two cases (UPSIT scores of 16 and 17) and in the control group individual UPSIT scores were all 23 or more. In the PD group, the group mean UPSIT score was 14.3 (95% CI 12.2, 16.3) and all subjects had individual UPSIT scores below 19 except one whose score was 24. In EOPD without parkin mutations the mean was 17.1 (95% CI 14.8, 19.4) and all subjects had individual UPSIT scores below 21 except one whose UPSIT score was 23. There was a significant difference in UPSIT scores between the healthy controls and the group of EOPD patients without PARK2 mutations (p<0.0001) and the group of PD patients (p=<0.0001). There were no significant differences between EOPD without PARK2 mutations and PD (p=0.80).

4.3.4 Conclusion

This study describes the first assessment of olfaction in parkin disease; olfactory function appears to be normal and therefore differs significantly from patients with EOPD who do not have PARK2 mutations and from PD where Lewy bodies have been reported in the olfactory bulb and tract with significant neuronal loss in the anterior olfactory nucleus (Daniel et al., 1992, Pierce et al., 1995).

Parkin is a protein that co-localises within actin filaments and is expressed in neuronal processes and cytoplasm of select neurones (Huynh et al., 2000). Expression of parkin mRNA in humans has been reported to be restricted to a number of regions in the normal brain (Solano et al., 2000). Our findings suggest that the ubiquitin-mediated proteolytic pathway involving the mutant parkin protein spares the olfactory neural networks.

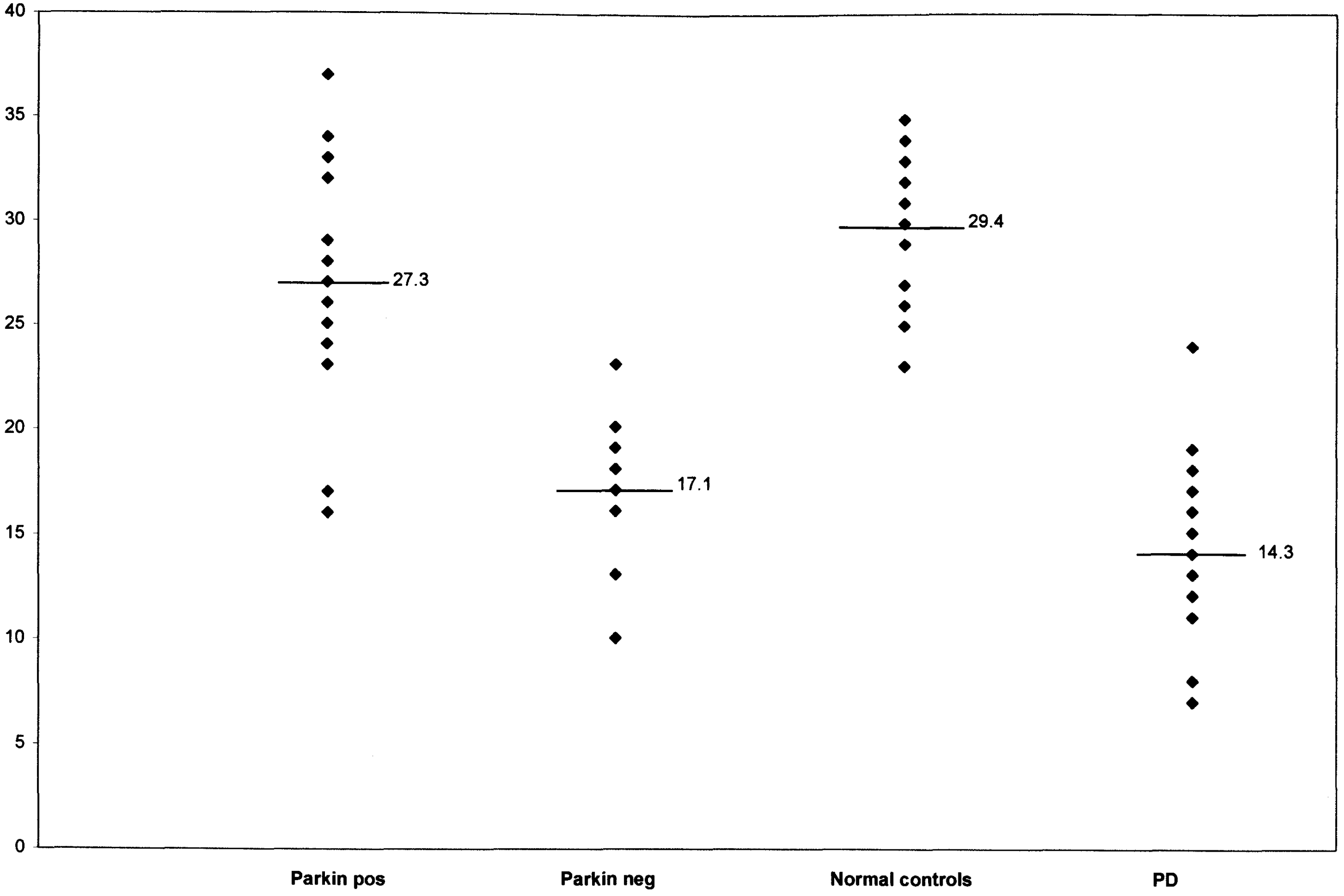
Neuropathological findings have been reported parkin; in two cases Lewy bodies were present in substantia nigra and locus coeruleus (Farrer et al., 2000, Chen et al., 2004). In remaining cases there was an absence of Lewy bodies and a severe and generalised loss of dopaminergic neurons from the substantia nigra pars compacta and locus coeruleus (Mori et al., 1998, Van de Warrenburg et al., 2001, Hayashi et al., 2000, Farrer et al., 2000). One brains showed a few neurofibrillary tangles and argyrophilic astrocytes in cerebral cortex and brainstem nuclei (Mori et al., 1998), another showed additional involvement of the substantia nigra pars reticulata (Van de Warrenburg et al., 2001), and the third showed neuronal loss in parts of the spinocerebellar system (Hayashi et al., 2000). Despite these rather incongruous findings the pathological lesions in parkin disease appears to be more restricted than in PD. The olfactory pathway has not been formally studied in parkin disease and our results underline the need for such neuropathological studies.

The methodology did not include screening the PD cohort (age of onset >50 years) for parkin mutations however parkin mutations in isolated cases of parkinsonism is rare. Moreover severe olfactory dysfunction is reported in PD (Doty et al., 1988); this was

Table 4.3.3 Clinical characteristics of the groups of subjects.

	Control subjects *(n=28)*	*Parkin positive subjects* *(n=17)*	*Parkin negative subjects* *(n=11)*	*IPD subjects* *(n=18)*
Mean age Onset (yrs)(SD)	-	23.4+/-8.0	33.4+/-12.9	58.1+/-5.4
Range of Age of Onset (yrs)	-	7-35	19-49	51-67
Mean age (yrs))(SD)	50.5 +/- 13.0	47.4 +/- 13.0	55.1+/-11.7	68.0+/-6.1
Disease Duration (yrs)(SD)	-	24.0+/-9.7	21.1+/-5.4	9.8+/-5.2
M/F	23/5	13/4	10/1	11/7
Smokers	6	5	1	1
History of Head injury	0	0	0	0

Figure 4.3.3
Scatter plot showing the University of Pennsylvania Smell Identification Test (UPSIT) scores in all groups. Abbreviations: parkin neg = parkin negative group; parkin pos = parkin positive group; PD = Parkinson's disease group. The larger values on the UPSIT score denote a greater degree of olfactory function. The bar refers to the median UPSIT value



not only confirmed in the cohort of PD patients in this study but this group also showed a significant difference in UPSIT scores from the parkin positive group.

Parkin disease has clinical and pathological features which may show varying degrees of overlap with PD. This is the first study of olfaction in parkin disease and the overall finding of normal olfaction supports existing neuropathological, genetic and functional imaging data suggesting that EOPD due to PARK2 mutations is a separate disease entity from Lewy body PD. Quantitative measures of olfaction may assist in distinguishing parkin disease from other forms of young onset levo-dopa responsive parkinsonism and PD.

5. ^{18}FLUORO-DOPA POSITRON EMISSION TOMOGRAPHY IN FAMILIAL FORMS OF PARKINSON'S DISEASE AND PARKINSONISM.

5.1 Introduction

At the time of this study, functional imaging using ^{18}F-dopa PET was a novel tool used to study the patterns of nigrostriatal dysfunction in Mendelian PD. This chapter describes work using ^{18}F-dopa PET to study patterns of nigrostriatal dysfunction in familial forms of parkinsonism and PD. All scans and analysis of data was performed at the MRC Cyclotron Unit, Clinical Sciences Centre, Imperial Colleague, Hammersmith Hospital, London and was supervised by Dr Paola Piccini.

The aims of the study were fourfold:

i) To assess progression of nigrostriatal dysfunction in an Irish kindred with parkin disease.

ii) To use ^{18}F-dopa PET to study unrelated, asymptomatic carriers of a single parkin mutation.

iii) To use ^{18}F-dopa PET to study patients and asymptomatic heterozygotes with autosomal recessive PARK6-linked, (PINK1) parkinsonism.

iv) To use ^{18}F-dopa PET in the British kindred with ADPD to identify presymptomatic nigrostriatal dysfunction for use in linkage analysis.

5.2 Progression of nigrostriatal dysfunction in an Irish kindred with Parkin Disease.

5.2.1 Introduction

In 1991 an Irish kindred with early onset levodopa-responsive parkinsonism (EOPD) was studied with ^{18}F-dopa PET (Sawle et al., 1992). At that time the four clinically affected siblings showed severely reduced ^{18}F-dopa influx in striatum. Moreover, putamen ^{18}F-dopa uptake was also mildly reduced in two asymptomatic members but normal in another asymptomatic sibling. Following the identification of mutations in PARK2, parkin, causing early onset parkinsonism in 1998 (Kitada et al., 1998), an affected individual from this kindred was shown to be a compound heterozygote, having a deletion in exon 8 (personal communication C Lucking) and a splice site point mutation in intron 5 (personal communication M Farrer). In this study ^{18}F-dopa PET was repeated 10 years later in all four siblings with EOPD. In addition, four asymptomatic relatives with a single mutant parkin allele and one with a normal genotype were also scanned. Two of the parkin carriers and the relative with a normal genotype had serial ^{18}F-dopa PET seven years apart.

The aims were to study the rate of disease progression in parkin disease in this kindred and to assess the presence and possible changes with time of subclinical nigrostriatal dysfunction in carriers of a single parkin mutation.

5.2.2 Methods

5.2.2.1 Patients

Ten members of this family underwent a standardised neurological examination. A retrospective review of medical records and video material taken at the time of the first scan was used to estimate the Unified Parkinson's Disease Rating scale (UPDRS) (Fahn et al., 1987) and the Hoehn and Yahr (H&Y) (Hoehn et al., 1967) score at the time of the first scan. Scores on the H&Y scale and the UPDRS scale in a practically defined 'off' state (12 hours after withdrawal of medication) were used to rate the degree of parkinsonian disability at the time of the second scan. All subjects gave informed consent on both occasions and the project was approved by the Joint Research and Ethics Committee of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, Queen Square, London UK.

5.2.2.2 PARK2 molecular analysis

5.2.2.2.1 Haplotype studies

Parkin haplotypes were assigned using chromosome 6q25-q27 DNA markers D6S1550, D6S305, D6S411, D6S253 (GDB accession ID: 641523, 63059, 237957, 62264 consecutively) using methods previously described in chapter 3.2. Haplotypes were manually constructed under the assumption of the minimum number of recombinations

5.2.2.2.2 Mutational analysis of PARK2, parkin

All living family members were screened for the exon 8 deletion and the exon 5 splice site point mutation by Mary Sweeney, Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London. The exon 8 deletion was detected using a semi-quantitative PCR method as previously described (Lucking, 2000). The PCR products were analysed on an ABI 377 automated sequencer and Genescan v 3.1.2. Genotyper v.2.5.1 software (Applied Biosytems) to determine the ratio of the peak heights of PCR products within the parkin gene used to detect the presence of the deletion. Exon 5 was amplified from genomic DNA by the PCR as previously described (Kitada et al., 1998). Both strands were sequenced using a Big Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing kit (Applied Biosystems, Foster City, CA), on an ABI 373 automated sequencer with the Sequence Analysis v.3.4.1 (Applied Biosytems) software.

5.2.2.3 PET scans

5.2.2.3.1 Scanning protocol

At the time of the first scan seven family members underwent ^{18}F-dopa PET on a CTI 931 scanner (CTI/Siemens, Knoxville, TN) with a reconstructed resolution of 1.5 cm as previously described (Sawle et al., 1992).

The second scan was performed on an ECAT 966 scanner (CTI/Siemens, Knoxville, TN) with a reconstructed resolution of 6mm. Following a transmission scan obtained with an external ^{68}Ge source, a mean dose of 129.5 MBq ^{18}F-dopa was injected as a bolus over 30 seconds and scanning was started at the onset of tracer infusion. Scanning began at the start of tracer infusion with 25 time frames over 95 min. Prior to emission data acquisition a transmission scan was performed with an external rotating positron source of ^{137}Cs to allow a measured attenuation correction to be performed.

All subjects gave prior written informed consent and were asked to stop their medication for 12 hours before the PET scan. Permission to perform these studies was obtained from the Ethics Committee of the Hammersmith Hospitals Trust, London, UK and from the Administration of Radioactive Substances Advisory Committee (ARSAC), UK.

5.2.2.3.2 Region of Interest Analysis

Image analysis of all scans for each subject was performed using Analyze software (version 7.5, BRU, Mayo Foundation, Rochester, Min., USA) on SUN Sparc Ultra computer workstations. ^{18}F-dopa uptake was expressed as an influx rate constant (Ki) and was calculated from caudate and putamen counts 25 to 95 minutes post injection using multiple time graphical analysis (MTGA) with occipital tissue counts as a reference tissue input function (Brooks et al., 1990). A standard ROI template was applied to parametric Ki images generated by in-house Kronos software (D Bailey) written in IDL image analysis software (Research Systems, Inc., Boulder, Co, USA): each putamen was sampled with an elliptical region 10x24 mm aligned along its axis and each head of caudate with a circular region diameter 10mm. All ROI's were placed by inspection with reference to the stereotactic atlas of Talairach and Tournoux (Talaraich, 1988). For each patient caudate and putamen ^{18}F-dopa influx rate constants (Ki) were measured.

Because two different scanners were used over the 10year period, ^{18}F-dopa uptake was expressed as a percentage of the normal mean in healthy controls scanned with the respective scanners.

On the first occasion 16 unrelated age matched controls (mean = 58.5±14.3 years, range = 28-75 years) scanned on the CTI 931 scanner were used as controls. For the second scan, 14 unrelated, age matched controls (mean = 54.6±13.9, range = 30-71 years) scanned on the ECAT 966 scanner with identical methods used for the parkin patients were used as controls. The controls reported no family history of parkinsonism and all had a normal neurological examination.

Seven patients with IPD, originally scanned on the 931 PET camera, with disease severity (evaluated on both clinical and ^{18}F-dopa striatum uptake ground) similar to the parkin group at the time of their first scan, were re-scanned on the 966 PET scanner after an interval of 5.04 (±1.73) years between scans.

For those subjects who had repeat scans the percentage annual rate of decline of ^{18}F-dopa Ki (r) was calculated using the formula: r = [(mean Ki for the group of healthy controls -subject Ki) / mean Ki of the group of healthy controls] / number of years between consecutive scans) x 100.

5.2.2.3.3 Statistical analysis

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

5.2.3 Results

5.2.3.1 Clinical findings

This parkin kindred originated from a small village in Southern Ireland (Figure 5.2.3). There was no known parental consanguinity and the parents did not have parkinsonism. The affected cases fulfilled the clinical diagnostic criteria of the Queen Square Brain Bank for PD except for the presence of a family history (Gibb et al., 1988). Four (one woman and three men) out of ten siblings were affected at the time of the study in 1990 and no further members of this kindred have subsequently developed the disease. The mode of transmission was consistent with autosomal recessive inheritance.

Mean age of onset of symptoms was 29 years (range 28-32 years). All affected siblings had a striking response to L-dopa therapy with levodopa-related dyskinesias after a mean interval of 2.4 years. Estimated mean clinical disease duration was 26 years (range 19-32 years) at the time of the second scan. The clinical presentation of the patients was comparable to that of juvenile-onset PD cases, however, currently their phenotype was indistinguishable from IPD with a common feature of severe resting leg tremor (abduction-adduction oscillations). Clinical features are summarized in Table 5.2.3.1. The following account provides more detail of each subject studied in this kindred (Figure 5.2.3)

Patient III.1

This 60 year old man reported initial symptoms of fatigue, lower limb pain and slowness from the age of 28 years with subsequent in turning of both feet. At the time of the first scan he was H&Y stage II with a UPDRS 'off' score of 39. Following a

10year interval, at the time of the second scan, disease duration was 32 years and he had now reached H&Y stage III and an UPDRS off score of 59. In the 'off' state he had a predominant abducting-adducting oscillatory tremor of both lower limbs at rest with asymmetrical bradykinesia and rigidity (left more than right). His posture was upright and he was unsteady on his feet. He had been receiving L-dopa for 14 years and continued to report a significant improvement taking 1000mg a day albeit with moderate inter dose dyskinesia and severe motor fluctuations. He was able to walk up to 3 miles at a time and maintain a job as a local handyman.

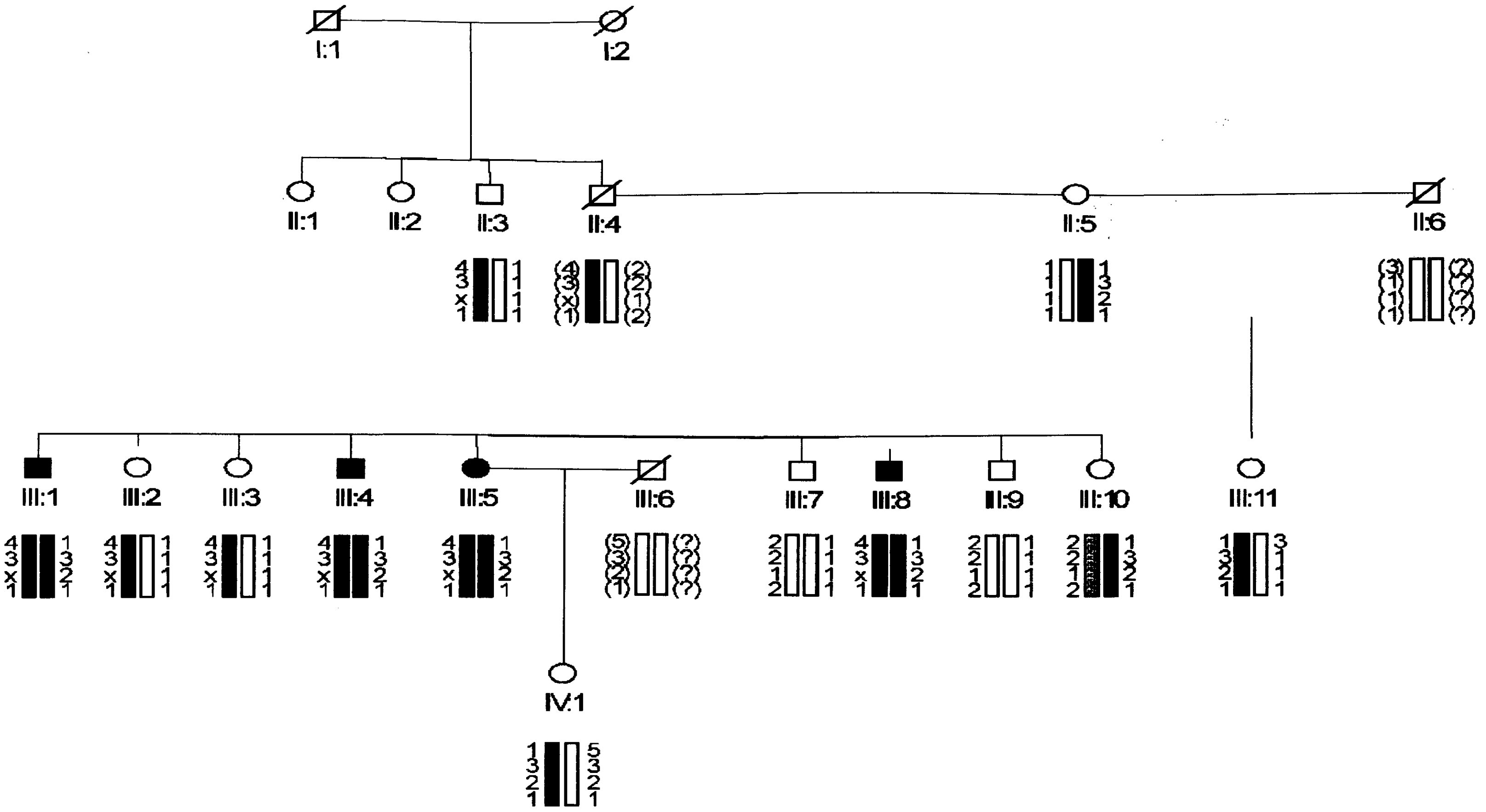

I:1
I:2
II:1
II:2
II:3
II:4
II:5
II:6
III:1
III:2
III:3
III:4
III:5
III:6
III:7
III:8
II:9
III:10
III:11
IV:1

Figure 5.2.3.1
Pedigree of the family with parkin disease with genotypes of DNA markers D6S305-0.04cM-D6S1550-1.35cM-D6S411-3.66cM-D6S253 (marker distances according to Genethon genetic maps). Haplotypes that segregate with parkin mutations are indicated in black such that the paternally inherited disease chromosome is indicated by 4-3-x-1 and the maternally inherited disease chromosome is indicated by 1-3-2-1. The position of the deletion on the paternal disease chromosome is indicated by an 'x'. Markers in parentheses were inferred on the basis of offspring data. Markers surrounded by question marks could not be phased with certainty.

Patient III.4

This 55year old man reported initial symptoms of slowness, right leg tremor and involuntary inversion of both feet from the age of 27 years. At the time of the first scan he was H&Y stage II with a UPDRS 'off'' score of 57. Following an 11year interval, at the time of the second scan, disease duration was 29 years and he had progressed to H&Y stage IV and a UPDRS 'off' score of 108. In the 'off' state he had a severe abducting-adducting oscillatory tremor of both lower limbs at rest with symmetrical bradykinesia and rigidity, postural instability and poor arm swing. At the time of the second scan he had been on L-dopa therapy for 23 years and continued to report significant improvement and dyskinesias with L-dopa, taking 1200mg a day. He was, however, no longer able to continue with employment as a landscape gardener; his disability had been exacerbated by lumbar canal stenosis such that he required walking sticks and at times, a wheelchair.

Patient III.5

This 54year old housewife reported symptoms of slowness, leg pain and leg tremor aged 31. At the time of the first scan she was H&Y stage II and UPDRS 'off' score was 42. After 11years, at the time of the second scan, disease duration was 19 years and she had progressed to H&Y stage IV and UPDRS 'off' score of 74. She had motor fluctuations with her 'off periods' with marked generalised stiffness, pain, bilateral resting leg tremor, shuffling gait and poor balance such that she was barely able to mobilise independently and perform activities of daily living. She had been on L-dopa for 11 years and continued to report a significant improvement from L-dopa taking 500mg a day but had developed motor fluctuations and dyskinesias.

Table 5.2.3.1 Clinical characteristics for each of the clinically affected subjects with parkin mutations and for a group of seven patients with idiopathic Parkinson's disease (IPD).

	III.1	III.4	III.5	III.8	mean ±SD	IPD (n=7) mean ±SD
age of onset	28	27	31	32	29.0 ±5.0	58.5 ± 4.8[1]
age at 1st scan	50	45	43	40	44.5 ± 4.2	64.7 ± 5.0[2]
disease duration at 1st scan (years)	22	18	12	8	15.0 ±6.2	6.20 ± 2.4[3]
interval between scans (years)	10	11	11	11	10.7 ±0.5	5.04 ± 1.7
H&Y First scan	2	2	2	2	2	2.79 ± 0.6[4]
H&Y Second scan	3	4	4	4	3.75 ±0.5	4.07 ± 0.6[5]

Wilcoxon test (parkin group vs IPD group) [1] p=0.0061, [2]p=0.0061, [3] p=0.0100, [4] p=0.1090, [5] p=0.412

Patient III.8

This 51year old former builder reported initial symptoms of bilateral leg tremor and involuntary inversion of the right foot from the age of 32. At the time of the first scan he was H&Y stage II with an UPDRS 'off' score of 59. He developed inter-dose painful dystonic cramps of the feet and severe chorea of the head, trunk and legs. Following an 11year interval, disease duration was 19 years at the time of the second scan and he had progressed to H&Y stage IV and a UPDRS 'off' score of 90. 'Off'' periods were associated with fatigue and distressing rest tremor of the legs and to a lesser degree the arms. Rigidity and bradykinesia were worse in the legs. At the time of the second scan he had been receiving L-dopa therapy for 18 years and continued to report a significant improvement, taking 1200mg a day.

None of the carriers of a single parkin mutation reported any symptoms. Individual IV.1 was first examined at the age of 19 years and then had ^{18}F-dopa PET when she was 24 and again when she was 30. At 19 years of age, individual IV.1 had a postural tremor of the right arm. At follow up, eleven years later, there was a postural tremor of both hands (right more than left), rest tremor of both legs and reduced arm swing on the right. Tone was normal with no evidence of bradykinesia, hypomimia or micrographia. Despite these extrapyramidal signs the UK Brain Bank diagnostic criteria for PD (Gibb et al., 1988) were not fulfilled.

Individual III.2, a housewife, had a normal examination in 1990 but at the time of the second scan had facial masking and reduced left arm swing.

Individual III.3, a housewife, had a history of diabetes, depression and schizophrenia that had been previously treated with chlorpromazine. Present treatment for depression included olanzapine, procyclidine and sertraline. Clinical examination at the time of the first scan was normal but at follow up there was a positive glabellar tap with normal tone, poor arm swing and mild paucity of facial expression with a bucco-lingual masticatory syndrome and stereotypies of the lower limbs.

The neurological examination of III.11 was normal.

Of those siblings with a normal genotype, III.7 had a normal neurological examination. Individual II.3, a 75year old paternal uncle, was not examined on the second occasion nor scanned on either occasion.

5.2.3.2 Molecular data

Haplotype analysis in the region of parkin confirmed linkage (Figure 5.2.3.1).

A deletion in exon 8 was identified in a paternal uncle II.3 and siblings III.1-5 and III.8. These cases were hemizygous for alleles at marker D6S411 confirming the presence of a deletion in exon 8 and segregating with the paternal haplotype 4-3-x-1. The second mutation, an intron 5 +2 T->A splice mutation, was detected in the mother, II.5, siblings III.1, III.4, III.5, III.8, III.10, III.11 and offspring IV.1 and segregated with the maternal haplotype 1-3-2-1. The clinically affected individuals were compound heterozygotes having an exon 8 deletion and an intron 5 +2 T->A splice mutation and shared heterozygous haplotypes 4-3-x-1 and 1-3-2-1.

Of the asymptomatic cases, three carried a paternally inherited parkin-associated haplotype (II.3, III.2 and III.3) and a detectable exon 8 mutation and one sibling (III.10), one half-sibling (III.11) and offspring (IV.1) inherited the maternal parkin-associated haplotype and a detectable intron 5 +2 T->A splice mutation. Siblings III.7 and III.9 did not inherit either of the parkin-associated haplotypes and did not have an exon 8 deletion nor an intron 5+2 T->A splice site mutation.

5.2.3.3 PET data

5.2.3.3.1 Patients

Mean values for ^{18}F-dopa caudate and putamen uptake, expressed as percentage reduction relative to the normal mean, at the first and second scan for the parkin and the IPD groups are shown in Table 5.2.3.3.1.

At the time of the first scan, percentage reductions below the normal mean in caudate ^{18}F-dopa uptake in the parkin and in the IPD groups were not significantly different (55±12 vs 43±5, p=0.109), similarly putamen percentage reductions below the normal mean were not significantly different in the two groups (64±4 in the parkin vs 58±8 in the PD patients, p=0.315) (Table 5.2.3.3.1).

Figure 5.2.3.3.1a shows the individual rates of decline in putamen ^{18}F-dopa uptake relative to the normal mean for the four parkin patients and the seven IPD patients.

The mean percentage rate of decline of putamen ^{18}F-dopa uptake relative to the normal mean was 1.47 (±05) per annum for the parkin group and 5.71 (±1.63) per annum for the IPD group. The rates of progression between these two groups were significantly different (p=0.0008) (Figure 5.2.3.3.1b).

The mean percentage rate of decline of caudate ^{18}F-dopa uptake relative to the normal mean was 1.72 (±1.7) per annum in the parkin group and 3.51 (±1.04) per annum in the IPD group. This difference was not statistically significant (p=0.885).

Within the parkin group the mean percentage rate of decline in caudate was not significantly different from that of putamen (1.72±1.7 and 1.47 ±05 respectively, p=0.16), while within the IPD group the mean percentage rate of decline was significantly slower in the caudate than in the putamen (3.51±1.04 and 5.71±1.63 respectively, p=0.006).

5.2.3.3.2 Asymptomatic subjects

Sibling III.7, who had a normal genotype, had normal caudate and putamen ^{18}F-dopa uptake (Table 5.2.3.3.2), which remained normal after 6 years.

Individually, two parkin carriers showed low normal levels of caudate Ki (>1.5 SD below the normal mean) and three carriers had a low normal putamen Ki (>1.5 SD below the normal mean) (Table 5.2.3.3.2).

As a group the four carriers had significantly reduced mean caudate and putamen [18F]-dopa uptake (0.0119±0.00047 and 0.0117 ± 0.00054 respectively) compared to mean caudate and putamen [18F]-dopa uptake of the healthy controls (0.0153±0.0026 and 0.0168±0.0031 respectively) ($p=0.0126$ and 0.009 respectively) (Figure 5.2.3.3.2).

Two of the carriers (III.3 and IV.1) were scanned on two occasions (after 9 and 6 years respectively). The rate of changes in caudate and putamen [18F]-dopa uptake per annum was 0.14% and 0.09%, respectively, in carrier III.3 and 0.73% and 0.74%, respectively, in carrier IV.1.

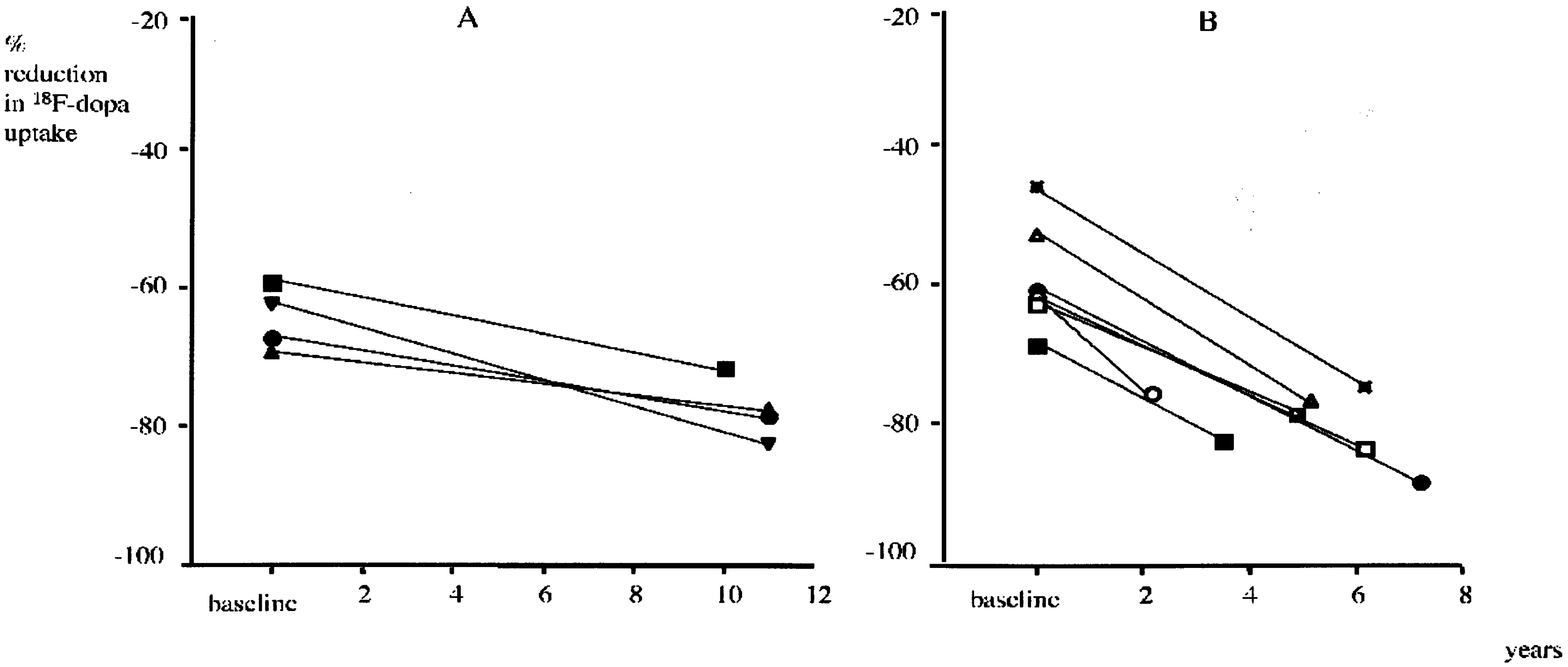


Figure 5.2.3.3.1a Individual rate of decline over time in ^{18}F-dopa putamen uptake in the four parkin patients (A) and in seven patients with idiopathic Parkinson's disease (B) matched for clinical disease severity at the time of first scan. Values are expressed as percentage (%) reduction of the mean of normal controls.

Figure 5.2.3.3.1b Mean percentage reductions per annum in ^{18}F-dopa caudate and putamen uptake in the group of parkin and in the IPD group. Patients were matched for disease severity at the time of first scan. Values are expressed as percentage reduction of the mean of normal controls. * Between groups Wilcoxon test.

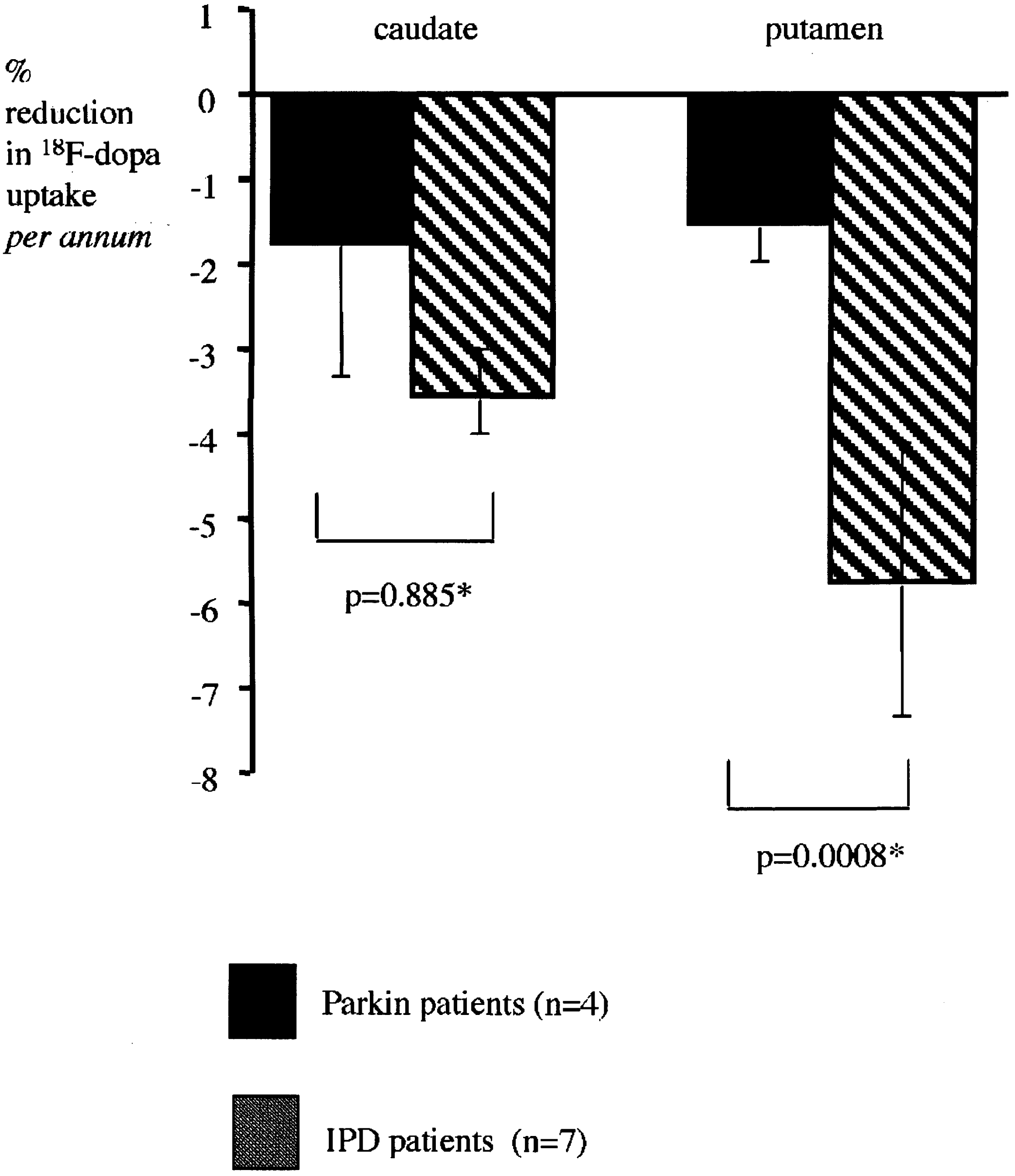


Table 5.2.3.3.1 Mean percentage reductions relative to the normal means in caudate and putamen ^{18}F-dopa Ki values in the four parkin patients and in the group of seven patients with idiopathic Parkinson's disease (IPD), at the time of the first (1) and the second scan (2) with the interval in years between scans.

	Caudate 1	Caudate 2	Putamen 1	Putamen 2	Interval
III.1	-40	-73	-59	-71	10
III.4	-50	-56	-67	-75	11
III.5	-68	-82	-69	-77	11
III.8	-62	-71	-62	-82	11
mean ±SD Parkin cases (n=4)	-55 ± 12	-69 ± 13	-64 ± 4	-76 ± 4	10.7 ±0.5
mean ±SD IPD cases (n=7)	-43 ± 5	-51 ± 5	-58 ± 8	-79 ± 5	5.0 ± 1.7

Table 5.2.3.3.2 Clinical, Genetic and ^{18}F-dopa PET characteristics of the asymptomatic members of the family at the time of the second scan.

	Controls (n=14) mean ±SD	III.2	III.3	III.11	IV.1	III.7
Age	50	59	58	69	30	52
Parkin carrier	-	yes	yes	yes	yes	no
Haplotype	-	paternal	paternal	maternal	maternal	neither
Exon 8 Deletion	-	yes	yes	no	no	no
Right caudate	0.0152 ±0.0028	0.0131	0.0127	0.0109§	0.0119	0.0127
Left caudate	0.0154 ±0.0025	0.0112§	0.0117	0.0115	0.0123	0.0155
Right putamen	0.0168 ±0.0032	0.0123	0.0114§	0.0113§	0.0102§	0.0154
Left putamen	0.0169 ±0.0032	0.0125	0.0123	0.0115§	0.0121	0.0164

Caudate and putamen ^{18}F-dopa values are expressed as Ki min^{-1}
§ denotes Ki influx constants at least 1.5 standard deviations below normal mean.

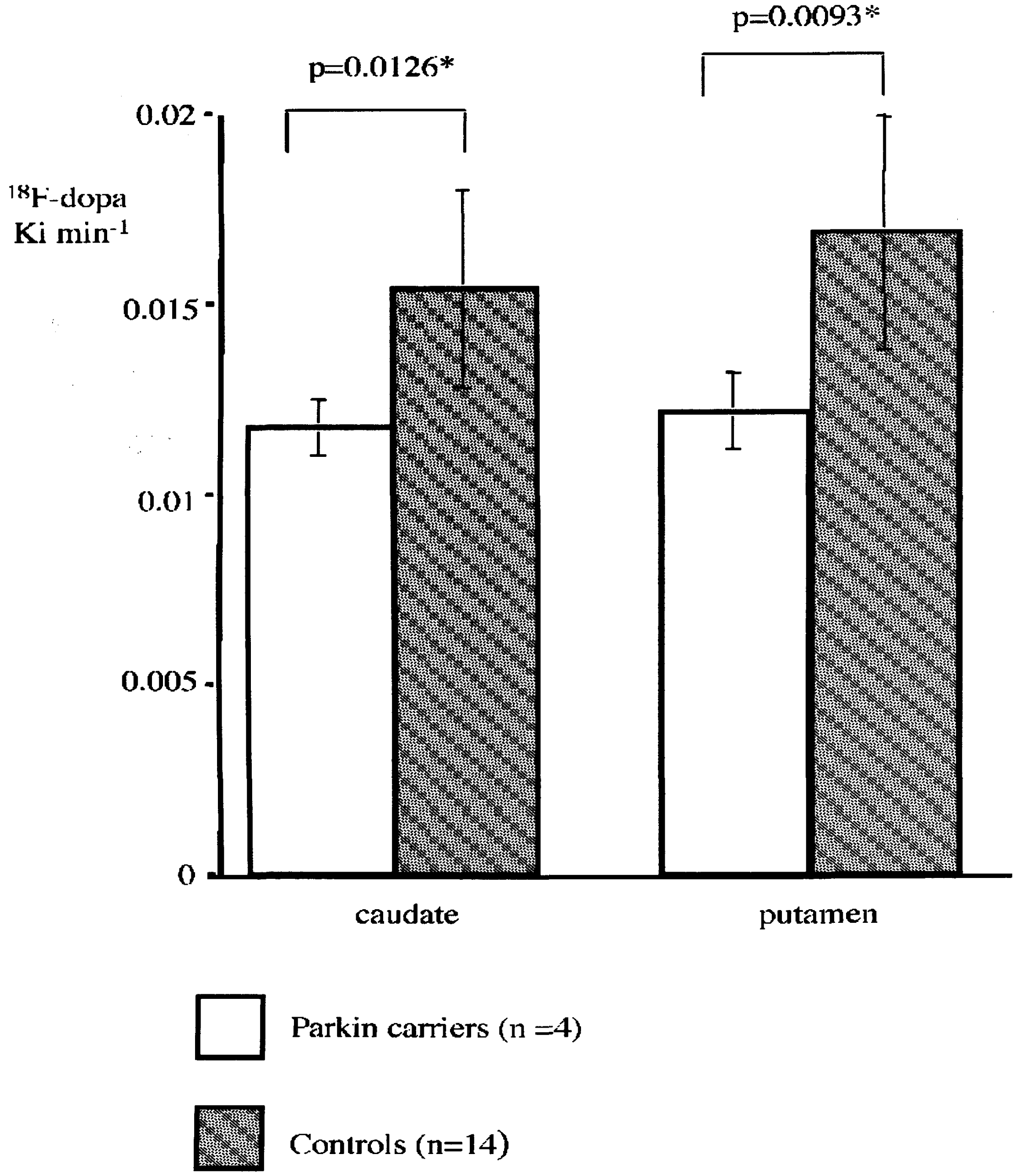


Figure 5.2.3.3.2
Mean caudate and putamen ^{18}F-dopa uptake in four parkin heterozygous carriers and in 14 age-matched, normal controls. Values are expressed as Ki min^{-1}. *Between groups Wilcoxon test.

5.2.4 Conclusion

Serial ^{18}F-dopa PET was used to measure over a ten-year period the rate of loss of dopaminergic function in clinically affected members of a kindred with parkin disease. The mean annual loss of putamen ^{18}F-dopa Ki relative to the normal mean was 1.48 % in parkin disease compared to 5.71 % in the group with IPD suggesting that disease progression in established parkin disease is slower. Findings for IPD patients are in agreement with previous studies which have reported a mean annual 6% loss of putamen ^{18}F-dopa storage relative to the normal mean or 12% relative to patient baseline (Morrish et al., 1996). Although the IPD group were not age matched with the parkin patients, their duration of clinical symptoms at the time of the baseline scan was not significantly different and they had a similar severity of disease to the parkin group, suggested by similar reductions of putamen ^{18}F-dopa uptake and motor scores. Similar severity and duration of disease at baseline rather then age are the critical factors when assessing rate of progression of Parkinson's disease in two groups of patients because ^{18}F-dopa striatal uptake per se is not influenced by age (Sawle et al., 1990; Eidelberg et al., 1993).

The rate of decline of putamen uptake amongst the symptomatic parkin patients was similar whilst there was some variability in the rate of decline of caudate uptake. However, mean caudate Ki deteriorated at approximately the same rate as mean putamen Ki in the parkin group whereas in the IPD group caudate dopamine function was relatively spared at baseline and deteriorated at a slower rate than putamen. The finding that nigrostriatal dysfunction in parkin disease progresses more slowly than IPD is in keeping with the clinical observation that cases of young onset parkinsonism can have a normal lifespan and suggests that neurodegeneration in parkin disease is an indolent process.

Intra-sibling variability of both Ki values and degree of parkinsonian disability amongst clinically affected cases may be due to the influence of other unlinked genes (modifier genes) and undefined environmental influences. Generally however, the pattern and course of the affected cases was remarkably similar.

Because of the lapse of time, more then 10 years, data from two different PET cameras were used. This methodology is not entirely rigorous this was compensated for by normalising patient data to control groups scanned with the respective scanners and by matching a group of IPD patients with similar disease severity and duration of clinical symptoms to that of the parkin group at the time of the first baseline scan. The sample size was small and confined to a single family such that the rate and pattern of nigral cell loss that was found may be specific to this family.

Dopaminergic dysfunction was also detected in the group of asymptomatic parkin carriers. In two carriers of a single parkin mutation, who were scanned 9 and 6 years apart respectively, there were no significant changes in ^{18}F-dopa uptake with time, similar to their sibling with normal genotype (III.7) and to that seen in normal volunteers (Morrish et al., 1996). This implies that either the two carriers have a fixed deficit that does not progress with time or that the rate of nigral cell death was too slow to be detected with PET over the time period. Alternatively, there may also be additional pathophysiological processes at the postsynaptic receptor not detectable by ^{18}F-dopa PET. The clinical development of mild signs of basal ganglia dysfunction between the two examinations is, however, intriguing and suggests incipient nigral cell dysfunction. Repeated observation with clinical examination and repeat scanning over time in a much larger cohort will be necessary to confirm these findings.

5.3 An ^{18}F-dopa PET study in unrelated, asymptomatic carriers of a single parkin mutation.

5.3.1 Introduction

Mutations in parkin, *PARK2* cause early onset parkinsonism and whilst the pattern of inheritance is presumed to be autosomal recessive; there was increasing interest in the risk of subjects with a single PARK2 mutation developing parkinsonism.

At the time of this study, functional imaging had been performed in asymptomatic parkin heterozygotes from just two families with parkin disease (Hilker et al., 2001, Irish kindred section 5.2 (Khan et al., 2002)). Subtle extrapyramidal signs had also been noted in parkin heterozygotes from both kindreds (Hilker et al, 2001, section 5.2) moreover there was a suggestion that carriers of a single mutation also had a susceptibility to psychiatric illness (section 4.2.3.2.9).

The aim of this study was to use ^{18}F-dopa PET to study 13 asymptomatic first-degree relatives with a single parkin mutation from eight unrelated patients with familial and isolated parkin disease in order to study subclinical dopaminergic dysfunction in a large cohort of unrelated parkin heterozygotes. This study included asymptomatic parkin heterozygotes from the Irish kindred reported in section 5.2.

5.3.2 Methods

5.3.2.1 Subjects

All subjects underwent a full and detailed neurological examination paying particular attention to the possible presence of mild extrapyramidal signs. All gave informed consent and the Joint Research and Ethics Committees of the National Hospital for Neurology and Neurosurgery, and the Hammersmith Hospitals Trust, London, UK approved the project. Permission to administer radiation was licensed by the Administration of Radioactive Substances Advisory Committee (ARSAC) UK.

5.3.2.2 Molecular Analysis

PARK2 PCR amplification, semiquantitaive PCR and sequence analysis was performed by Elizabeth Graham, Laboratory Technician, Department of Molecular Neurosciences, Institute of Neurology, Queen Square, London as previously described.

5.3.2.3 PET Scans

5.3.2.3.1 Scanning protocol

All subjects were scanned on an ECAT HR++ 966 scanner (CTI/Siemens, Knoxville, TN) with a reconstructed resolution of 4mm and an axial field of view of 24 cm using a protocol that is outlined in 5.2.2.3.1.

5.3.2.3.2 Region of Interest Analysis

Two regions of interest (ROIs) were defined using a protocol that is outlined in 5.2.2.3.2. The caudate to putamen ratio (C/P ratio) of each subject was calculated using the formula r = mean caudate Ki / mean putamen Ki. The ROI analysis was performed blinded to the status of both the group of parkin heterozygotes and the group of control subjects.

5.3.2.3.3 Statistical parametric mapping

Statistical parametric mapping (SPM99) was investigated by Dr Christof Scherfler, MRC Cyclotron Unit, Clinical Sciences Centre, Hammersmith Hospital, London. SPM99 was used to localize significant differences in dopaminergic function between the parkin carrier and healthy control group. SPM was performed using SPM99 software (Wellcome Dept of Cognitive Neuroscience, Institute of Neurology, Queen Square, London, UK) implemented in Matlab 5.3 (Mathworks Inc., Sherborn, Mass., USA). The obtained datasets allowed categorical comparisons of mean voxel [^{18}F] dopa K_i^0 values between groups of parkin carriers and control subjects.

Fourteen unrelated, age and sex-matched controls (mean age 54.6 +/- 13.9, range 30-71 years) were scanned with a similar protocol to the parkin heterozygotes. All normal controls reported no family history of parkinsonism and had a normal neurological examination.

5.3.2.3.4 Statistical analysis

The non-parametric Wilcoxon test was used for all comparisons between groups of patients and controls.

5.3.3 Results

5.3.3.1 Clinical findings

A total of 13 parkin heterozygotes (subjects 1-13, Table 5.3.3.1) from eight unrelated parkin kindreds (Families 1-8, Table 5.3.3.1) were studied. Eight subjects were men and the remaining five were women. Seven were first-degree relatives of isolated cases and six were first-degree relatives of familial cases (two or more clinically affected) of an index patient with parkin disease. The mean age of the group was 52.0 ± 13.0 years and the age range was 30-69 years. None reported symptoms of parkinsonism.

Four heterozygote parkin gene carriers had subtle extrapyramidal signs (tremor, reduced arm swing - Table 5.3.3.1), which did not fulfil the UK PDS brain bank criteria for IPD (Gibb, 1988). The remainder had a normal examination (Table 5.3.3.1). The clinical characteristics of all subjects are summarised in Table 5.3.3.1.

5.3.3.2 PET findings

Individual mean (left –right averaged) ^{18}F-dopa influx constant, Ki, values obtained with ROI analysis for the thirteen parkin heterozygotes and the mean of the control group and

Table 5.3.3.1 Parkin mutations and clinical characteristics of the asymptomatic parkin heterozygotes.

isolated or familial index patient	Relation	Parkin mutation	Subject	Age at scan	Sex	Examination findings
1 / isolated	father	exon 7 867c>t	1	48	male	normal
2 / isolated	sister	Exon12 1390g>a	2	34	female	normal
2 / isolated	father	Exon 3 del	3	54	male	normal
2 / isolated	mother	Exon12 1390g>a	4	51	female	normal
3 / isolated	sister	Exon 2 del	5	64	female	normal
4 / familial	daughter	Exon 3 del	6	46	female	normal
5 / isolated	father	Exon 5 del	7	60	male	normal
6 / isolated	father	Exon 5 del	8	68	male	poor arm swing
7 / familial	son	Exon 7or 12	9	30	male	normal
8 / familial	sister	In5T>A	10	69	female	normal
8 / familial	sister	Exon 8 del	11	60	female	poor arm swing/facial masking
8 / familial	sister	Exon 8 del	12	58	female	poor arm swing/ facial masking
8 / familial	daughter	In5T>A	13	30	female	postural tremor hands, rest tremor both legs, poor arm swing

Table 5.3.3.2a Individual ^{18}F-dopa striatal Ki (left-right averaged) values of the parkin heterozygotes and in the group of 16 normal volunteers.

	^{18}F-Dopa Ki (min^{-1})	
Subject	caudate	putamen
PARK2 heterozygotes		
1	0.0116 §	0.0115 §
2	0.0101 *	0.0096 +
3	0.0108 *	0.0126 §
4	0.0094 *	0.0133
5	0.0154	0.0179
6	0.0137	0.0143
7	0.0122	0.0121 §
8	0.0146	0.0136
9	0.0121	0.0113 §
10	0.0111 §	0.0111 §
11	0.0111 §	0.0114 §
12	0.0125	0.0134
13	0.0122	0.0124 §
Mean	0.0120[a]	0.0126[b]
(±SD)	(0.0021)	(0.0029)
Controls (n = 14)		
Mean	0.0153	0.0169
(±SD)	(0.0026)	(0.0031)

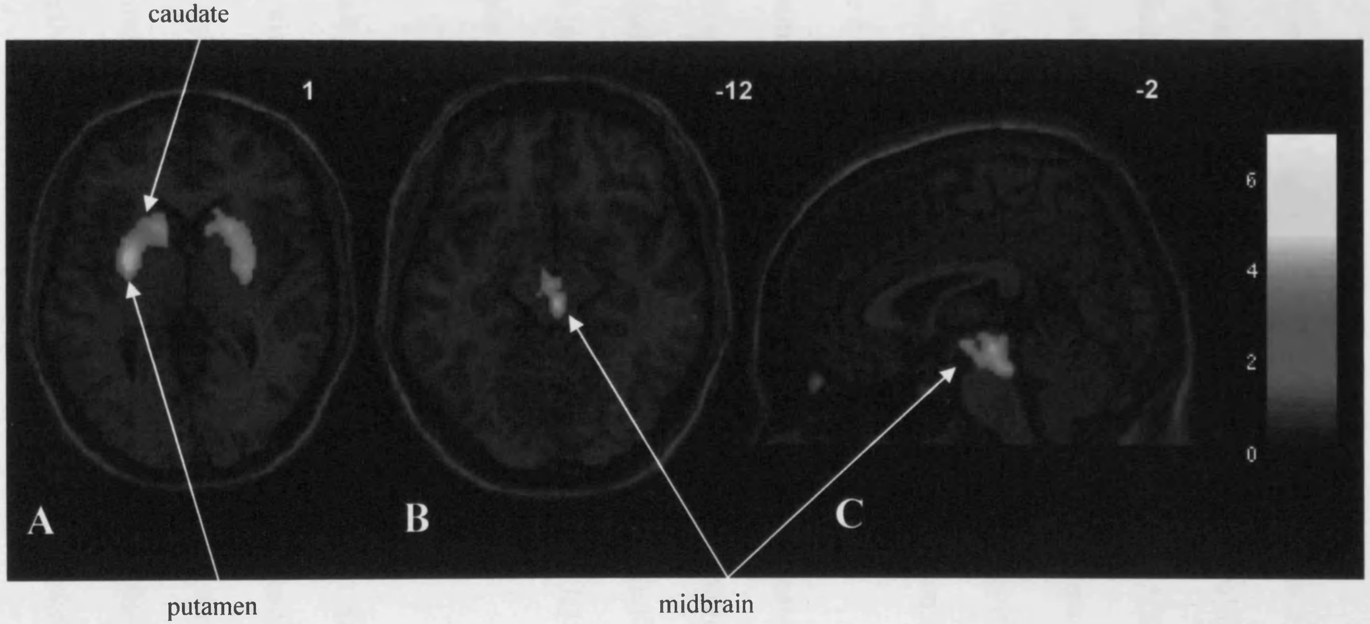
Wilcoxon test (PARK2 heterozygotes group vs controls)
[a] p = 0.008
[b] p = 0.002

SD = standard deviation
§ = 1.5 SD below normals
* = 2.0 SD below normals
+ = 2.5 SD below normals

Table 5.3.3.2b Between-group SPM findings showing the locations of significant decreases of ^{18}F]-dopa Ki in heterozygous parkin carriers compared to control subjects.

^{18}F-dopa $K_i$min^{-1} Area	Talairach co-ordinates			Z score	P values (corr.)	Height threshold
	x	y	z			
Decreases in heterozygous parkin carriers compared with control subjects						
Left putamen	-24	0	0	4.87	0.0001	0.001
Right putamen	22	13	3	4.23	0.0001	
Dorsal midbrain	2	-22	-9	4.37	0.0500	
Right caudate	22	15	3	4.23	0.0001	0.01
Left caudate	-15	14	7	3.43	0.0001	
Ventral midbrain	2	-12	-8	3.14	0.0001	

Figure 5.3.3.2 SPM{Z} transverse and sagittal maximum intensity projection maps rendered on to a stereotactically normalised MRI scan, showing areas of significant decreases in caudate and putamen (A) and midbrain (B, axial slice; C, sagittal slice) of [^{18}F]-dopa Ki^0, uptake in patients heterozygous for parkin mutations compared with healthy control subjects. Numbers in A and B correspond to the Z co-ordinate, number in C corresponds to the X co-ordinate in Talairach space. With kind permission from Dr Christoph Scherfler, Clinical Sciences Centre, MRC Cyclotron unit, Hammersmith Hospital, London



are shown in Table 5.3.3.2a. As a group, mean parkin heterozygote caudate Ki values (0.0120±0.002) and mean putamen Ki values (0.0126±0.003) were significantly reduced by 23% and 27% below the group means of control caudate Ki values (0.0153±0.003, $p=0.0008$) and putamen Ki values (0.0169±0.003, $p=0.0022$) (Table 5.3.3.2a).

This was confirmed by SPM analysis (Figure 5.3.3.2). When compared to a group of healthy controls, asymptomatic parkin carriers showed significant bilateral decreases in ^{18}F-dopa uptake in the caudate and putamen ($p<0.001$, corrected; Fig 5.3.3.2) and caudate ($p<0.01$, corrected; Fig 5.3.3.2). Significant decreases in ^{18}F-dopa Ki were also observed in the dorsal and ventral midbrain regions in heterozygote carriers compared to controls (z score4.4; $p<0.01$;x=2mm; y=-24mm; z=-9mm,Tailarach space) (Figure 5.3.3.2; Table 5.3.3.2b).

Individually, eight parkin heterozygotes had a low normal putamen Ki greater than 1.5 SD below the normal mean and six had a caudate Ki greater than 1.5 SD below the normal mean); three subjects (2, 3 and 4) had caudate Ki values 2SD below normal mean; subject 2, though without abnormal neurological findings, had a mean putamen Ki value 2.5 SD below the control mean (Table 5.3.3.2a).

5.3.4 Conclusion

This is the first in vivo study of dopamine terminal function in a large number of unrelated asymptomatic parkin heterozygotes; there was a significant reduction in mean caudate, putamen and midbrain ^{18}F-dopa uptake in these subjects with a similar uniform pattern to that reported in patients with clinical parkin disease (Section 5.2 (Khan et al., 2002), Scherfler et al., 2004). As ^{18}F-dopa is also taken up by serotonergic and noradrenergic as well as dopaminergic neurons in midbrain areas our results would suggest that aberrant, ubiquitin ligase-mediated cell function in parkin heterozygotes is, as in clinically affected parkin patients, not just restricted to dopaminergic neurones though the reduction is less severe than that seen in patients with parkinsonism due to parkin mutations (Hilker et al., 2001, section 5.2 (Khan et al., 2002), Scherfler et al., 2004). Individual findings in the parkin heterozygotes are of interest. Subject 13, though asymptomatic, was found to have a postural tremor of both upper limbs at the age of 19 (Sawle et al., 1991). After 11 years of follow up, when the patient was 30 years old and still asymptomatic, ^{18}F-dopa PET showed severe nigrostriatal dopaminergic dysfunction and clinical signs had progressed with the development of mild resting tremor in the legs and reduced arm swing. Three subjects (subjects 2, 3 and 4) individually showed significant reductions in caudate dopamine storage compared to controls but had an entirely normal neurological examination. The disparity between the absence of clinical findings and significant nigrostriatal dopaminergic dysfunction compliments findings reported for patients with early onset parkinsonism associated with mutations in parkin (section 5.2) where severe reductions in striatal ^{18}F-dopa are found. It suggests that even if significant loss of dopamine cell function occurs early it may then progress slowly enough to allow compensatory mechanisms to develop. It is known that in idiopathic PD there is increased dopamine turnover in surviving striatal terminals (Bezard et al., 1998). Additionally, recent reports have shown increased pallidal dopamine storage in early PD cases (Whone et al., 2001). These mechanisms, in addition to altered production of non-dopaminergic neurotransmitters, may all operate to maintain motor status. Interestingly, there was no correlation between age of the heterozygote and the degree of dopamine terminal dysfunction. Subjects 2 and 4 with the lowest caudate influx constants, shared a point mutation in exon 12, causing Gly430Asp amino acid substitution in the RING-finger motif at the carboxy terminus of the protein, which may play an important role in cell growth and differentiation. Single base pair substitutions may have an overall greater loss of function compared to deletions (and absent protein) because the intact but inactive protein may compete for substrate and deleteriously affect parkin-mediated cellular pathways.

These findings suggest that haploinsufficiency where a single mutant allele reduces normal protein function by up to 50% (Strachan, 2000) results in a reduction of ubiquitin ligase activity, which may not be sufficient for normal nigrostriatal activity. This would imply that parkin's ligase activity is dosage sensitive and that sufficient relative levels of enzyme are required for interaction with other gene products of the ubiquitin-conjugating pathway. The effects of modifying genes particularly influence those cases in which the phenotype is dependent on haploinsufficiency and environmental factors (Strachan, 2000). An alternative explanation is a dominant negative effect (Strachan, 2000), the non-functional mutant parkin polypeptide physically interferes with the function of the normal polypeptide suggesting that dimerization or oligomerization of the protein is requisite for normal function. Moderate reduction in protein function caused by either haploinsufficiency or a dominant negative effect may produce nigrostriatal damage in heterozygous carriers.

This would suggest that having a single mutant parkin allele could prove to be a risk factor for the development of subclinical disease. Moreover four of our subjects were 'manifesting heterozygotes' implying that perhaps different parkin mutations impart different degrees of susceptibility to developing clinical signs. Repeated observation over time will be necessary to confirm whether these cases go on to develop full-blown parkin disease.

An alternative explanation is that the ^{18}F-dopa PET changes observed are of no clinical significance, however, the manifestation of subtle extrapyramidal signs in three parkin carriers (one of whom, however, had a history of neuroleptic exposure) suggests that these individuals may well be 'manifesting heterozygotes' and raises the possibility that they may be at risk of developing parkin disease. Severe reduction however in ubiquitin ligase activity in homozygotes may produce the recessive condition, which results in marked nigrostriatal dysfunction and the clinical disease. One other explanation of these findings is that they reflect differences in the development of the dopaminergic system in parkin heterozygotes rather than subclinical decline: a study of post-synaptic D2-receptor binding and the functional status of dopaminergic transmission would seek to clarify this. However, there are now described cases of late onset parkin disease with only a single mutant allele (West et al., 2002) and a recent genome screen on idiopathic PD patients (Oliveira et al., 2003) has identified 17 PD patients with mean age of onset of 49.2 years who were heterozygote for parkin mutations (12 of them carrying mutations in exon 7), suggesting that heterozygous mutations especially those lying in exon 7 may act as susceptibility alleles for late-onset form of PD.

5.4 An ^{18}F-dopa PET study of autosomal recessive PARK6-linked parkinsonism.

5.4.1 Introduction

A locus for autosomal recessive parkinsonism was linked to chromosome 1p35-p36, PARK6, in a family from Sicily, the Marsala kindred by colleague Dr Enza Maria Valente (Valente et al., 2001). Since the completion of this study the gene has been cloned and mutations have been identified in PINK1 (Valente et al., 2004). There is considerable overlap of the clinical phenotype with that of idiopathic 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 (ARJP) are absent in PARK6 (Valente et al., 2002).

At the time of this study neither post-mortem data nor *in vivo* studies on the function of the nigrostriatal dopaminergic system were available for PARK6-linked parkinsonism or asymptomatic heterozygotes.

^{18}F-dopa PET was used to study two families: the Marsala kindred (family 1) and an unrelated Italian family, the Abruzzo kindred (family 2), in whom disease was linked to PARK6 (Valente, 2002). 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.

5.4.2 Methods

5.4.2.1 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 members (Valente et al., 2001). The other two patients (one man and one woman) were from the Abruzzo kindred, family 2, which included 43 family members (Bentivoglio et al., 2001).

5.4.2.2 Asymptomatic PARK6 heterozygotes

Three subjects were carriers of a single mutant PARK6 haplotype: two were from family 1 (VI.24, VII.8, (Figure 1, Valente et al., 2001) and one subject was from family 2 (III.6, Figure 2, (Bentivoglio et al., 2001). None of the carriers reported any symptoms or showed any signs of PD.

All subjects underwent a standardised neurological examination. Scores in a practically defined 'off' state on the Unified Parkinson's Disease Rating scale (UPDRS) and the Hoehn and Yahr (H&Y) were used to rate the degree of parkinsonian disability. All subjects gave informed consent on both occasions and the project was approved by the Joint Research and 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 (ARSAC) UK.

5.4.2.3 Scanning protocol

All subjects were scanned using a protocol previously described in section 5.3.2.3.1.

5.4.2.3.1 Region of Interest Analysis.

Four regions of interest (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 10x12mm the posterior part of the dorsal putamen with an elliptical region 10x12mm and the entire dorsal putamen with an elliptical region 10x24 mm aligned along its axis. All ROI's were placed by inspection with reference to the stereotactic atlas of Talairach and Tournoux (Talairach et al., 1988) on five contiguous transaxial slices. For each patient head of caudate, anterior, posterior and entire putamen ^{18}F-dopa influx rate constants (Ki) was computed using linear graphical Patlak analysis with an occipital reference tissue input function (Brooks, 1990). The caudate to putamen ratio (C/P ratio) of each subject was calculated using the formula r = mean caudate Ki / 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, and fourteen unrelated, age and sex matched controls (mean age 54.6 +/- 13.9, range 30-71 years) 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.

5.4.2.3.2 Statistical analysis

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

5.4.3 Results

5.4.3.1 PARK6-linked parkinsonism: clinical findings.

These cases all fulfilled the clinical diagnostic criteria of the Queen Square Brain Bank for probable PD except for the presence of a family history (Gibb et al., 1988). H&Y and UPDRS motor scores for each subject at the time of the scan are summarised in Table 5.4.3.1. 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. Clinical characteristics are summarised in Table 5.4.3.1.

Table 5.4.3.1. Clinical characteristics and ^{18}F-dopa striatal Ki values in the four PARK6 patients and in the group of eight patients with Parkinson's disease.

		Clinical characteristics					^{18}F-dopa Ki (min^{-1})				
		age of onset (yrs)	age at scan	DD (yrs)	H&Y	UPDRS motor score	caudate	putamen	anterior putamen	posterior putamen	C/P index
PARK6 patients	VI.7*	45	76	31	3.5	73	0.0051	0.0025	0.0026	0.0025	2.3
	VI.23*	38	47	9	2	22	0.0059	0.0022	0.0025	0.0014	1.5
	IV.8§	38	41	3	1	5	0.0041	0.0031	0.0029	0.0029	1.2
	IV.7§	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[3]	0.0027	0.0028[4]	0.0024	1.8[5]
	(±SD)	(5.3)	(16.3)	(12.1)	(1.0)	(28.9)	(0.0008)	(0.0004)	(0.0005)	(0.0007)	(0.4)
PD patients	mean	53.0[1]	67.3[2]	11.0	3.3	38.4	0.0102	0.0042	0.0060	0.0026	2.8
	(±SD)	(4.5)	(5.5)	(5.1)	(0.7)	(15.0)	(0.0016)	(0.0019)	(0.0015)	(0.0014)	(0.6)

* Family 1 (Figure 1,[1]) § Family 2 (Family 2, Figure 2,[3])
DD = disease duration. H & Y = Hoehn and Yahr staging[5], UPDRS = Unified Parkinson's disease rating scale[5]
C/P index = caudate/putamen index.
Wilcoxon test (PARK6 patient group vs PD group)[1] p<0.001, [2] p=0.04, [3] p=0.01, [4] p=0.03, [5] p=0.03.

Figure 5.4.3.2 Mean caudate, anterior and posterior putamen ^{18}F-dopa uptake in the group of patients with PARK6-linked parkinsonism and in the group of PD patients matched for disease severity. Values are expressed as Ki min^{-1}. * p=0.01, § p=0.03

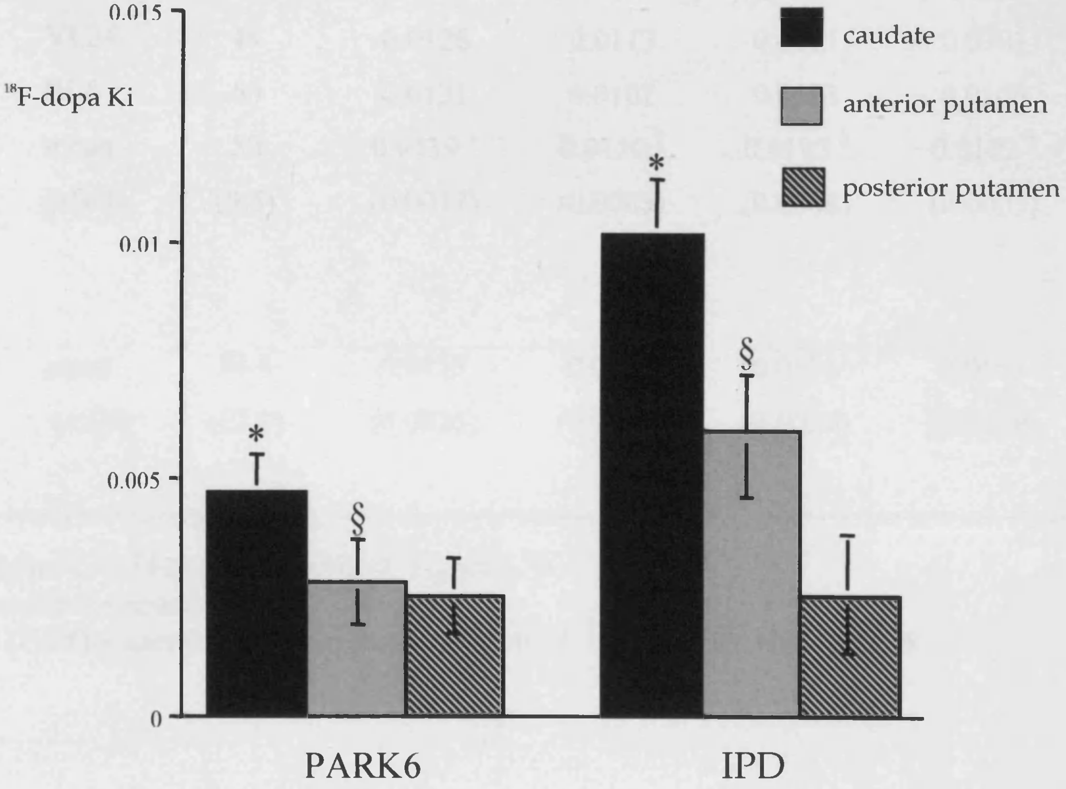


Table 5.4.3.3 ^{18}F-dopa striatal Ki values in the three PARK6 carriers and in the group of 14 normal volunteers.

			^{18}F-dopa Ki (min^{-1})				
			Caudate		Putamen		C/P index
		age		Putamen	Anterior putamen	Posterior putamen	
	VII.8	41	0.0099	0.0111	0.0115	0.0102	0.90
	VI.24	49	0.0126	0.0113	0.0131	0.0101	1.10
PARK6 carriers	III.6	60	0.0131	0.0107	0.0123	0.0105	1.20
	mean	50	0.0119 [1]	0.0110 [2]	0.0123 [3]	0.0102 [4]	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)

* Family 1 (Figure 1,[1]), § Family 2 (Family 2, Figure 2,[3])
C/P index = caudate/putamen index.
Wilcoxon test (PARK6 carrier groups vs controls) [1]p=0.03, [2]p=0.01, [3]0=0.01, [4]p=0.05

5.4.3.2 PARK6-linked parkinsonism: PET findings.

Regional mean ^{18}F-dopa Ki values obtained for the four clinically affected PARK6 patients and the PD group are shown in Table 5.4.3.1 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 cohort compared with the PARK6 group ($p=0.03$ and $p=0.01$ respectively) (Table 5.4.3.1). Additionally, the mean caudate/putamen ratio in PARK6 patients was significantly lower compared to the PD group ($p = 0.02$) (Figure 5.4.3.2)

5.4.3.3 Asymptomatic PARK6 heterozygotes: PET findings.

Individually, all three PARK6 carriers had low normal putamen Ki (>1.5 SD below the normal mean) and one of them (VII.8, figure 1, (Valente et al., 2001)) also had a low normal caudate Ki >1.5 SD below the normal mean. As a group the three carriers had mean caudate and putamen ^{18}F-dopa uptake significantly reduced in comparison to the normal group mean ($p=0.03$, $p=0.01$, for caudate and putamen respectively) (Table 5.4.3.3).

5.4.4 Conclusion

This is the first in vivo study of dopamine terminal function in PARK6-linked parkinsonism and asymptomatic carriers of a single PARK6-linked haplotype. Although the group 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 due to 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 (Bernheimer et al., 1973). This data suggests that the neurodegenerative process in PARK6-linked parkinsonism involves the nigra more uniformly and may well have different neuropathological features from PD.

Individual PET findings of the PARK6 patients are of interest. First, patient IV.8, with only three 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, there was 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 (Hilker et al., 2001, section 5.2 (Khan et al., 2002)), another recessive form of early-onset parkinsonism, and suggests that significant dopamine cell loss occurs early in life in patients with recessive parkinsonism. Disease may then 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 (Bezard et al., 1998) Additionally, a recent report showed increased pallidal dopamine storage in early PD cases (Whone et al., 2001) .These mechanisms plus altered production of non-dopaminergic neurotransmitters may all operate to maintain motor status. The PARK6 sample size was small, however, and we cannot exclude the fact that that the pattern of nigrostriatal dopamine dysfunction we found in these carriers may be specific to the two families.

Nigrostriatal dysfunction was also found in three members 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 previously been reported in asymptomatic co-twins of PD patients (Piccini et al., 1999) and at-risk adult members of unrelated kindreds with familial parkinsonism (Piccini et al., 1997) one third of who subsequently developed clinical disease. These 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 (Hilker et al., 2001, section 5.3 (Khan et al., 2002).

A possible molecular explanation for the findings of abnormal nigrostriatal dysfunction in heterozygous PARK2 and PARK6 carriers could be either haploinsufficiency (Strachan, 2000) 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 (Strachan, 2000) where the non-functional 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 sub-clinical 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.

5.5 An ^{18}F-dopa PET study of asymptomatic members of the Lincolnshire kindred.

5.5.1 Introduction

The work described reported here was performed on unaffected members of the Lincolnshire kindred. This took place prior to a genome-wide screen and hence carrier status of a disease-causing locus in each unaffected family member that was scanned was unknown.

Whilst this kindred had over twenty members reported to have PD, DNA was only available from six affected subjects thus limiting the power of a genome wide study in an autosomal dominant kindred.

The purpose of this study was to use ^{18}F-dopa PET to identify nigrostriatal dysfunction as a surrogate marker of 'presymptomatic disease' in clinically unaffected subjects in the Lincolnshire kindred. This would assist in extending the phenotype to include subclinical disease thus increasing the numbers of the 'affected' and the power in linkage analysis.

5.5.2 Methods

^{18}F-dopa PET scans were performed and analysed by myself using methods previously reported (section 5.2.2.3). Ethics Committees of the National Hospital for Neurology and Neurosurgery, and the Hammersmith Hospitals Trust, London, UK approved the project. Permission to administer radiation was licensed by the Administration of Radioactive Substances Advisory Committee (ARSAC) UK.

5.5.3 Results

Five subjects in whom the clinical examination was normal were scanned. III.3 (aged 77), III.4 (aged 73), III.7 (aged 71), III.21 (aged 77), IV.7 (aged 42) (Figure 2.3.2); none of these subjects had reduction of ^{18}F-dopa uptake values of 2 standard deviations or more compared to a group of 14 controls (Table 5.5.3).

Table 5.5.3 Individual ^{18}F-dopa striatal Ki (left-right averaged) values of asymptomatic members of the British kindred with autosomal dominant PD (Figure 2.3.2) and in a group of 14 normal volunteers.

	^{18}F-Dopa Ki (min^{-1})	
Subject	caudate	putamen
III.3	0.0122	0.0118
III.4	0.0126	0.0119
III.7	0.0130	0.0163
III.21	0.0129	0.0134
IV.7	0.0137	0.0134
Mean	0.0129	0.0134
(±SD)	(0.0005)	(0.0018)
Controls (n = 14)		
Mean	0.0153	0.0169
(±SD)	(0.0026)	(0.0031)

The controls reported no family history of parkinsonism and all had a normal neurological examination.

5.5.4 Conclusion

Prior to commencing the molecular characterisation of the Lincolnshire kindred, five family members without clinical manifestation of the disease underwent ^{18}F-dopa PET scans to detect preclinical nigrostriatal dysfunction. Imaging was normal. These findings were substantiated by subsequent haplotype analysis (Figure 3.31) and mutation analysis (Paisan-Ruiz et al., 2004) that confirmed that these subjects did not possess a mutant allele.

This novel approach combining two diverse scientific tools of functional imaging and molecular genetics failed to identify pre-symptomatic disease in this kindred however it has been successful elsewhere. This methodology was applied to a large Spanish family with typical, autosomal dominant PSP where disease status was defined according to the clinical and positron emission tomography data in unaffected subjects; PSP was successfully linked to a 3.4 cM region on chromosome 1q31.1 (Ros et al., 2005).

6. POPULATION BASED ASSOCIATION STUDIES

The use of linkage analysis in large nuclear families has identified some of the genetic components of PD. However despite the wealth of data generated by the scientific community a definite and replicable susceptibility locus has not been identified in the idiopathic PD population. There are a number of reasons for this discussed in section 1.2.1.1. Population based approaches in PD have included linkage analysis, affected-sibling pair analysis and association studies; each has had a variable degree of success (Tan et al., 2000).

Work described in this section describes two association studies. The first was an attempt to replicate a positive association with disease and the second study employed a candidate gene approach to identify if an association existed in PD populations.

Alpha synuclein / Apolipoprotein E

6.1.1 Introduction

Kruger and colleagues investigated polymorphisms in two plausible candidate genes that contribute to neurodegeneration: the α-Synuclein gene, α-SYN on chromosome 4 and Apolipoprotein E (Kruger et al., 1999). Immunohistochemistry has demonstrated that α -synuclein is a component of the LB (Spillantini et al., 1997), synucleins have apolipoprotein-like secondary structure (Clayton et al., 1999) and the overlap in clinicopathology of PD and AD may imply an overlap of susceptibility to age-modulated disease by the Apoε 4 allele. Kruger and colleagues identified a significant association of alleles in the promotor polymorphism of α-synuclein (NACP) as well as closely linked DNA markers D4S1647 and D4S1628 and the Apoε 4 allele with PD in a German population (Kruger et al., 1999). A combination of Apoε 4 / NACP genotype was found to increase susceptibility to PD 12 fold (Kruger et al., 1999).

In an attempt to replicate these results same NACP polymorphism, Chromosome 4p markers and Apolipoprotein E was genotyped in a much larger PD population (305 cases) in which the majority of PD cases were histopathologically proven and a larger number of closely matched control samples (335). PD samples used also included a cohort of familial cases as well as a larger number of young onset cases. A novel polymorphism of the intron 4 region of α-SYN (IN4) was also studied.

6.1.2 Methods

6.1.2.1 Patients

The study population consisted clinical and pathological PD cases. Clinical cases evaluated by a neurologist, diagnosed PD based on the presence of two or more of the cardinal features (tremor, rigidity and bradykinesia) and an absence of other signs unrelated to PD. All subjects gave informed consent and the study was approved by the Joint Research and Ethics Committees of the National Hospital for Neurology and Neurosurgery and Institute of Neurology, Queen Square, London. Brain tissue from PD cases was obtained from the Queen Square Brain Bank at the Institute of Psychiatry, London, UK. DNA extracted from control brains was obtained from the MRC Neurochemical Pathology Unit, Newcastle General Hospital Newcastle, UK. DNA from all clinical samples was obtained from the diagnostic service laboratory, Neurogenetics Section, Institute of Neurology, Queen Square London. DNA was extracted from brain tissue and blood leucocytes with use of conventional methods previously described.

Control cases were selected to closely match age and sex of the PD population and were samples of brain tissue with no abnormal histopathology or clinical cases from the National Hospital for Neurology and the Institute of Neurology, Queen Square, London. Control clinical cases used did not have parkinsonism.

6.1.2.2 α-synuclein promotor polymorphism (NACP)

The allele status of the dinucleotide marker in the promotor region α-SYN, was determined by PCR with the flanking primers:

Rep1 (5' GCAATGGAGTAGACAAAAGGATGG 3') and

Rep2 (5' CTACATGACTGGCCCAAGATTAA 3'). Reactions were performed in a volume of 20 µl containing 10 ng of genomic DNA, 0.2mM each dNTP,10% PCR buffer II, 2.5mM MgC12, 10pmol each primer, 0.5 U Amplitaq Gold. GeneAmp 9700 thermal cyclers were used: samples were subjected to a denaturation step of 95 degrees C for 3 minutes followed by 35 cycles 95 for 15 seconds, 55 for 20 seconds and 70 for 50 seconds. A final extension of 10 minutes at 70 was specified. was Samples were run on 4% polyacrylamide gels with an ABI 377 automated sequencer equipped with GENESCAN (v3.1). Alleles were sized and alleles assigned with GENOTYPER (v2.5). Three different alleles were identified 257 bp, 259 bp and 261 bp. Positive control samples were obtained from Kruger and colleagues allele 1, 2 and 3 corresponded to our alleles 261, 259 and 257 respectively.

6.1.2.3 Markers D4S2460, D4S423, D4S1578, D4S1628, 4S1647 in coding region of the α-synuclein gene.

Tetranucleotide (D4S1628, D4S1647) and dinucleotide (D4S1578, D4S423, D4S2460) repeat markers were amplified by using primer sequences and PCR conditions described by the genome database (accession ID: D4S2460, GDB:424508 ; D4S423, GDB:62948; D4S1578 (substituted D4S2422 from the original paper), GDB:245064, D4S1628, GDB:686793, D4S1647, GDB:691159). Samples were run and alleles were sized and assigned as described for NACP. Marker D4S2460 showed 8 different alleles sized as 175, 177,179,181,183,185,187,189 bp. Marker D4S423 showed 12 different alleles, D4S 1578 showed 10 different alleles, D4S1628 showed 4 different alleles sized as 146,150,154,158 bp (directly corresponding to positive controls from Kruger and colleagues 149, 153,157 and 161bp) and D4S1647 showed 6 different alleles 132, 136, 140,144,148,152 with identical size/mobility to those alleles typed in the original study.

6.1.2.4 α-synuclein Intron 4 Polymorphism (IN4)

Allele status for this TC rich polymorphism identified approximately 0.9kb downstream of the exon 4/intron 4 boundary, was determined by PCR using the flanking primers IN4F: 5'ATTCTTCTCACCTCTGGTATTC3' and IN4R: 5' TTAAAGGTGAATAACACTTGGC 3'. Reactions were performed in a volume of 20 μl containing 10 ng of genomic DNA, 0.2mM each dNTP, 10% PCR buffer II, 2.5mM MgCl2, 10pmol each primer, 0.5 U Amplitaq Gold and 5% DMSO. The remainder of the PCR conditions, sizing and assignation of alleles was as described for NACP. Four alleles were identified and sized as 256bp, 266bp, 288bp and 366bp.

6.1.2.5 Apolipoprotein E Genotyping

Apoliprotein E genotyping was performed using methods described (Wenhamet al., 1991) by Dr Rohan De Silva, Reta Lila Weston Institute of Neurological Studies, Royal Free and University College Medical School London.

6.1.2.6 Statistical Analysis

Statistical analysis was performed by Dr Adrian Mander and Dr David Clayton, MRC-Biostatistics Unit, Institute of Public Health, Forvie Site Cambridge UK. Allele frequencies rather than genotype frequencies were used to improve power however this method assumed Hardy Weinberg Equilibrium (HWE) which was tested and was found to be present. The HWE assumption was used for each marker using a kappa based test. The allelic association test is a chi-squared test on the m by 2 table where m is the number of alleles. When m is large for highly polymorphic markers the table may be sparse this may invalidate the asymptotic sampling distribution of the Pearson chi squared statistic. The table was therefore analysed using Monte Carlo methods as described (Sham et al., 1995). Linkage Disequilibrium was tested between pairs of markers using an EM algorithm to resolve phase (Chiano et al., 1998, Gambaro et al., 2000).

6.1.3 Results

The study population consisted of 305 unrelated PD cases (see 6.1.3a for details). Of this, 170 samples were brain tissue with pathologically proven PD: samples showed depletion of pigmented neurons in the substantia nigra and locus coeruleus and the presence of Lewy bodies. There were no glial cytoplasmic inclusions or additional pathology to account for parkinsonism. The remaining 135 PD samples were clinical cases of which 66 were familial cases having a sibling and/or an affected parent with PD. The 305 samples were also subdivided in to young onset PD (YOPD; mean age onset 44 years -+6 SD) of which there were 89 cases and late onset PD (LOPD; mean age onset 63 years) of which there were 206 cases.

330 control samples of which 175 were brain tissue with no abnormal histopathology were used. The remaining 155 cases were clinical cases: 52 cases of hereditary sensory motor neuropathy and 103 cases of spinocerebellar ataxia. Control clinical cases used did not have parkinsonism.

In this population X^2 analysis showed that there is no significant association between any allele of any of the markers typed and PD (Table 6.1.3b). In particular the independent association of disease with alleles of NACP (261bp), D4S1628 (158bp) or D4S1647 (140bp) observed by Kruger and his colleagues, was not replicated, despite the allelic distribution in the control sample being comparable to those found by Kruger and his colleagues. Allele frequencies of the complete sample are shown in Table 6.1.3c. Linkage disequilibrium was not detected between pairs of Chromosome 4 markers, NACP or IN4.

By analysing a total of 305 PD samples and 330 age and sex matched controls no difference in the allele distribution of ApoE was identified (X^2= 1.09, p-value= 0.05) and the Apoε 4 allele did not have a significant distribution in the YOPD group (X^2= 4.21, p- value= 0.14) or the FPD group (X^2= 0.357, p-value= 0.77). Of particular significance was that the finding of a 12 fold increased risk of PD with a combined apoε 4 / NACP allele sized 261 genotype was not replicated (p-value=0.96).

6.1.4 Conclusion

In this sample of 335 controls and 305 PD cases a susceptibility to disease with NACP allele, D4S1628, D4S1647 and Apoε 4 nor the combined NACP 261bp/Apoε 4 genotype was not observed in contrast to Kruger and colleagues (Kruger et al., 1999). The α-synuclein gene is a plausible candidate gene for PD risk due to its pre-synaptic localisation, deposition of the NAC protein in the Lewy body (Spillantini et al., 1997) and the segregation of missense mutations in the α-synuclein gene in two autosomal dominant PD kindreds (Polymeropoulos et al., 1997, Kruger et al., 1998). Synuclein proteins have an apolipoprotein-like structure: sharing amphipathic class A2 helices. The class A2 helix mediates apolipoprotein-like exchange and may interact with lipids and account for the early identification of synucleins as synaptic vesicle-associated proteins (Clayton et al., 1999). It is for these reasons that that this study attempted to replicate the findings of Kruger and colleagues.

Searching for population association can be a powerful method of identifying disease susceptibility loci (Strachan T, Read A, 1999). However this requires associations to be confirmed by larger study numbers with stringent standardisation of controls samples. Failure of replication does not necessarily render the original result false but there are several possible explanations for the discrepancy between two comparable groups including population stratification, statistical artefact, small sample sizes, different phenotypes, poorly matched controls and the biological credibility of the gene-allele- phenomenon-disease association (Strachan T, Read A, 1999, Gambaro 2000).

In this study the majority of PD cases are pathologically proven: the relevance being

Table 6.1.3a Characteristics of PD cases and Controls

Characteristics	PD cases	Controls
Number	305	330
Age (mean years +/- SD)	61 6SD	63 7SD
Male (%)	55	53
Familial/nonfamilial	66/239	NA
YOPD/LOPD	89/206	NA
Histopathological cases	170	175
Clinical cases	135	155

Table 6.1.3b Chi squared analysis of all markers used on Controls and PD cases

Marker	X^2	p-value
D4S2460	3.72	0.89
D4S423	13.3	0.365
D4S1578	15.55	0.09
D4S1628	3.47	0.39
D4S1647	3.97	0.615
NACP	5.10	0.26
IN4	1.00	0.8
ApoE	1.10	0.505

Table 6.1.3c Distribution of alleles of NACP, D4S1628 and D4S1647 in Control and PD cases

Allele Frequency

NACP	251	259	261
PD cases	0.261	0.655	0.084
Controls	0.251	0.694	0.055

D4S1628	146	150	154	158
PD cases	0.059	0.328	0.510	0.102
Controls	0.046	0.286	0.547	0.123

D4S1647	132	136	140	144	148	152
PD Cases	0.164	0.028	0.180	0.312	0.231	0.085
Controls	0.194	0.028	0.181	0.332	0.185	0.080

	265	266	288	366	
PD Cases		0.490	0.198	0.069	0.242
Controls	0.474	0.187	0.077	0.262	

that up to 25% of clinical cases of PD have an absence of typical histopathology (Hughes et a., 1992). There may be differences in linkage disequilibrium patterns between populations from the United Kingdom and Germany however recent work has shown that this may not be so significant (Eaves et al., 2000, Taillon-Miller et al., 2000). This study used a much larger sample size and stringent statistical analysis. Additionally the power of both this study and that of Kruger and his colleagues will be lower due to the low frequency of the NACP susceptibility allele and apoε 4 in the population. A definitive answer to the role of these candidate genes in susceptibility to PD will require significantly larger sizes of the order of 1000 cases with at least as many controls (Gambaro et al,, 2000) and a dense linkage disequilibrium map of single-nucleotide polymorphisms (SNP's) courtesy of the Human Genome Mapping Project (Collins, 1998).

6.2 Association between Angiotensin Converting Enzyme gene, *ACE* and Parkinson's disease.

6.2.1 Introduction

The angiotensin converting enzyme (ACE), encoded by gene dipeptidyl carboxypeptidase 1 *[DCP1]* also known as ACE gene, catalyses the cleavage of two amino acids from angiotensin I, converting it into the physiologically active peptide angiotensin II, which controls fluid and electrolyte balance and systemic blood pressure. The activity of the enzyme has been reported to be altered in regions of the brain in patients with Huntington's disease, Schizophrenia and AD moreover it has been identified as a normal constituent of cerebrospinal fluid (Zubenko et al., 1985). There are several reasons why *ACE* may be considered a candidate gene in the pathogenesis of PD. Firstly, Kehoe and co-workers (Kehoe et al., 1999) reported an association between AD and the insertion allele (I allele) of an insertion/deletion (I/D) polymorphism in intron 16 of the *DCP1/ACE* gene: the overlaps in clinicopathology of PD and AD may imply overlap in susceptibility to disease. Subsequent work by the same group, genotyping several single nucleotide polymorphisms in *ACE* in five independent populations revealed strong association with Alzheimer's disease with a haplotype containing the *Alu* insertion allele (Kehoe et al., 2003). Secondly, the regional distribution and activity of the ACE enzyme has been studied in normal human brain. The highest activity was found in hypothalamus, then (in order) caudate nucleus, substantia nigra, medulla oblongata and cerebral cortex (Tani et al., 1991); remarkably these represent the areas of the brain in which typical histopathological findings are identified in PD. Moreover, work has indicated that ACE contributes to the metabolism of amyloid β peptide in AD by inhibiting cytotoxity by preventing aggregation, deposition and fibril formation of amyloid β peptide and thus reducing susceptibility to AD (Hu et al., 2001). It could be hypothesized that ACE has a similar role in preventing protein aggregation in normal brain and that aberrant ACE activity allows insoluble fibrils (such as alpha synuclein, ubiquitin, parkin, DJ-1, PINK1) to aggregate and contribute to pathology and hence disease in PD.

Thirdly, there are high concentrations of the enzyme in the nigrostriatal pathway and basal ganglia (Zubenko et al., 1986) and in PD, levels of nigrostriatal angiotensin II receptors are markedly decreased (Allen et al., 1992). Fourthly, *in vivo* and *in vitro* experiments on rat striatum have shown that angiotensin II stimulates the release of dopamine (Simonnet et al., 1979, Dwoskin et al., 1992). Lastly, an example of a practical application of identifying a disease-causing allele; PD patients treated with perindopril, (an ACE enzyme inhibitor) reported faster onset and prolonged 'on' periods and a reduction in peak dose dyskinesias (Reardon et al., 2000).

This study used the intron 16 I/D polymorphism of the *DCP1/ACE* gene (Kehoe et al., 1999) which arises due to an insertion of a 287-base pair Alu repeat sequence, resulting in three genotypes (DD/II homozygotes and ID heterozygote (Rigat et al., 1992) to study the relationship between the *DCPI/ACE* gene and susceptibility to PD.

6.2.2 Subjects and Methods

6.2.2.1 Subjects

The first cohort (cohort A) studied consisted of 275 unrelated PD cases. Pathological confirmation was obtained for 147 samples (mean age of onset 61.7 years ± 7 SD) from the UK Queen Square Brain Bank for Neurological Diseases and the MRC Brain Bank, Institute of Psychiatry, London, UK. All showed severe depletion of pigmented neurones in the substantia nigra and locus coeruleus and the presence of Lewy bodies. There were no glial cytoplasmic inclusions or additional pathology to account for parkinsonism. The remaining 128 diseased clinical samples fulfilled the PDS brain bank criteria for the diagnosis of PD (Hughes et al., 1992). Of the diseased clinical subjects, 56 were familial (having a sibling and/or an affected parent with PD and 72 cases were EOPD samples (early onset parkinsonism age of onset <50 years, mean age of onset 42.7 years ± 7 SD). A second cohort (cohort B) of 186 PD cases were ascertained from the Cambridge Brain Repair Centre. All samples were clinical cases (mean age of onset 60.9 years ± 11.1 SD) that fulfilled the Queen Square brain bank criteria for the diagnosis of PD except for the presence of family history in 13 cases (Hughes et al., 1992). 29 were cases with early onset parkinsonism (mean age of onset 43 years ± 5.1 SD). A third and independent cohort of PD samples (cohort C) consisted of 264 unrelated PD cases. Pathological confirmation was obtained for 95 samples from the Queen Square Brain Bank. The remaining 170 diseased clinical samples fulfilled criteria for the diagnosis of PD (Hughes et al., 1992).

Controls samples were selected to closely match age and sex of the PD population. A total of 487 controls were used, of which 127 were from the brain tissue with no abnormal histopathology, from the MRC Neurochemical Pathology Unit, Newcastle, UK. 207 were healthy clinical cases from the Institute of Psychiatry, Denmark Hill, London. These subjects were UK residents, aged over 75 years and were recruited for the MRC Trial of Assessment and Management of Elderly People in the Community (Liolitsa et al., 2002). The remaining 153 were diseased controls used in section 6.2.1.2 of another association study.

All subjects gave informed consent. The project was approved by the Joint Ethics committee of the National Hospital for Neurology and Neurosurgery (NHNN).

6.2.2.2 Molecular Analysis

DCPI/ACE genotypes were constructed using established methods (Rigat et al., 1992). Each sample was genotyped three times to reduce genotyping error and scoring was blinded to the previous result. Genotyping of the *ACE* Insertion (I)/ Deletion (D) was performed as originally described (Rigat et al., 1992). The allele status was determined by PCR with the flanking primers: 5'-CTG GAG ACC ACT CCC ATC

CTT TCT-3' and 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3. The annealing temperature was 56°C and product sizes were 190bp and 478bp.

To ensure no mistyping of *ACE* I/D genotypes occurred an extra insertion-specific PCR step (Kehoe et al., 1999) was performed with flanking primers: 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3' and 5'-TCG GCA GCC CTC CCA TGC CCA TAA-3'. The annealing temperature was 56°C and product size was 335bp.

In addition we did not identify genotyping errors using identical methods to study 33 trios of parents and one offspring (data not shown).

All samples were electrophoresed in 1.6% agarose gels and were visualised under a UV transilluminator.

6.2.2.3 Statistical analysis

Statistical analysis was performed by Dr Danae Liolitsa, Department Molecular Neuroscience, Institute of Neurology, Queen Square, London using the SPSS software for Windows, release 10.0. Genotype or allele frequencies and percentage estimates for the control and PD groups were calculated using the SPSS database analysis programme. Chi-square analysis was carried out using the software EpiInfo (version 6) to examine the two groups for compliance with the Hardy-Weinberg equilibrium. Phenotypes and genotypes were recoded as appropriate and this resulted in many dummy variables that were entered into a logistic regression test. Binary logistic regression analysis was used to calculate odds ratios (OR) and the associated confidence intervals (CI) for the relationship of ACE genotypes with PD.

6.2.3 Results

In all control groups (Table 6.2.3a & 6.2.3b), there was no significant deviation from Hardy-Weinberg equilibrium. *ACE* genotype and allele frequencies of our control population (29.9, 49.7 and 20.4 percent respectively for the DD, ID and II genotypes) was similar to controls in previous reports (Kehoe et al., 1999, Lindpaintner et al., 1995) including one using 2340 controls subjects (Lindpaintner et al., 1995).

In the first PD population used, the heterozygous ID genotype was more common in the PD cohort than the homozygous DD and II genotypes of the *DCPI /ACE* gene, compared to controls and this difference was significant by logistic regression analysis (OR = 1.39, 95% confidence interval, 1.00 to 1.91; p = 0.033) (Table 6.2.3b). Separate subgroup analysis of the ID genotype compared to DD and II genotypes in which the case patient was defined with pathology only resulted in a significant difference compared to controls (OR = 1.55, 95% confidence interval, 1.03 to 2.30; p = 0.029) (Table 6.2.3b).

The association was replicated in an independent cohort of 264 PD cases (third cohort of both pathological and clinical cases) (p = 0.014) but not in another group of 186 clinical cases (OR = 1.03, CI = 0.72-1.48, p = 0.34).

A separate analysis using the dominant or recessive model for the DD or II genotypes did not show a significant effect (data not shown). Similarly, no significant relationships were found for the allele-specific effects on the phenotypes.

Table 6.2.3a Genotype and allele frequencies and percentage estimates for the I/D *ACE* polymorphism in PD cases and controls

	PD group				**Control group**			
	1st cohort N = 275	**2nd cohort N = 186**	**3rd cohort N = 264**	**Total N = 725**	**1st control N = 207**	**2nd control N = 127**	**3rd control N = 153**	**Total N = 487**
***ACE* genotypes**	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
DD	68 (24.7)	52 (28.0)	63 (23.8)	183 (25.2)	64 (30.9)	36 (28.3)	44 (28.8)	144 (29.6)
ID	159 (57.8)	94 (50.5)	156 (59.0)	409 (56.4)	101 (48.8)	65 (51.2)	68 (44.4)	234 (48.0)
II	48 (17.5)	40 (21.5)	45 (17.0)	133 (18.3)	42 (20.3)	26 (20.5)	41 (26.8)	109 (22.4)
***ACE* alleles**	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
D	295 (53.6)	198 (53.8)	282 (53.4)	775 (53.4)	229 (55.3)	137 (53.9)	156 (50.9)	522 (53.6)
I	255 (46.4)	174 (46.2)	246 (46.6)	675 (46.6)	185 (44.7)	117 (46.1)	150 (49.1)	452 (46.4)

Table 6.2.3b Genotype and allele frequencies and percentage estimates for the I/D *ACE* polymorphism in PD cases and controls and main effects from logistic regression analysis (OR, odds ratio; CI, confidence interval). All comparisons were made with total controls as seen in table 6.2.3a.

	1st cohort		**2nd cohort**	**3rd cohort**		**Total**
	Total	**Pathological**	**Total**	**Total**	**Pathological**	
	N = 275	**N = 147**	**N = 186**	**N = 264**	**N = 95**	**N = 725**
***ACE* genotypes**	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
DD	68 (24.7)	33 (22.4)	52 (28.0)	63 (23.8)	27 (28.4)	183 (25.2)
ID	159 (57.8)	89 (60.5)	94 (50.5)	156 (59.0)	54 (56.8)	409 (56.4)
II	48 (17.5)	25 (17.0)	40 (21.5)	45 (17.0)	14 (14.8)	133 (18.3)
2 degrees of freedom	χ^2= 6.8	χ^2= 7.05	χ^2= 0.84	χ^2= 8.42	χ^2 = 3.48	χ^2 = 8.24
	P = 0.033*	P = 0.029*	P = 0.34	P = 0.014*	P = 0.17	P = 0.016*

6.2.4 Conclusion

This study has identified a genetic variation at the *DCP1/ACE* locus that predisposes to PD. Moreover it is most significant in the histopathologically proven cohort suggesting that the *ACE* locus predisposes to parkinsonism associated with Lewy body pathology. Patients that are heterozygous for the insertion/deletion polymorphism of the *DCP1/ACE* gene have an increased risk of developing PD. This association is independent of the DD genotype which has been significantly associated with cardiovascular and cerebrovascular disease (Cambien et al., 1992); diseases which are 2.5 times higher in PD (Ben-Sholomo et al., 1995).

This study identified a heterozygous rather than either of the homozygous states as a susceptibility allele; why this is the case is unclear. It could be hypothesized that in the heterozygous state, an abnormal interaction between dissimilar protein domains or dimers exerts a detrimental effect on microfibrillar assembly or protein function that is not present with identical protein domains in the homozygous state. An example of a deleterious, intronic heterozygous mutation has been reported in Ullrich congenital muscular dystrophy. Here an intronic, heterozygous deletion of the COL6A1 gene results in a 33 amino acid deletion close to the triple helical domain important for dimer formation resulting in the secretion of abnormal tetramers exerting a strong dominant negative effect (Pan et al., 2003).

The *DCP1/ACE* insertion/deletion polymorphism has a highly reproducible effect on variation of plasma ACE protein levels (Keaveny et al., 1998) supporting a role for a gene in which phenotypes may be influenced by ACE activity. This association is well established in different populations (Tiret et al., 1993, McKenzie et al., 1995, McKenzie et al., 2001) and follows an additive pattern with the DD and II genotypes being associated with high and low ACE levels, respectively while the ID genotype is associated with intermediate levels (Rigat et al., 1990). The molecular mechanism by which the intronic *ACE* I/D polymorphism exerts its effects on plasma ACE levels is not yet known (McKenzie et al., 2001).

An alternative explanation is that the Alu element in *DCP1/ACE* is in absolute linkage disequilibrium with another sequence either within *ACE* itself, such as the coding region or the promotor, or in another gene closeby. A study showed that the Alu element in *ACE* is in absolute linkage disequilibrium with seventeen varying sites in the gene only two of which were coding (Rieder et al., 1999).

This study employed identical methods to that used to find a significant association of the insertion allele of ACE with Alzheimer's disease (Kehoe et al., 1999); results that were replicated using up to date methodology; Kehoe and co-workers confirmed that haplotypes using SNPs extending across *DCP1/ACE* are associated with the disease (Kehoe et al., 2003).

Population based genetic association studies can be a powerful method of identifying disease susceptibility loci (Strachan and Reid 2000). However an association requires confirmation in large study numbers in an independent cohort (Bird et al., 2001). This study confirmed an association in an independent cohort of PD samples but this did not extend to a third population of cases, all of which were clinical cases. The discrepancy may be due to a number of factors; up to 25% of clinical subjects with PD do not have characteristic histopathology at autopsy (Hughes et al., 1992). Isolated and familial cases of L-dopa responsive parkinsonism that fulfil the PDS brain bank criteria may in fact not have PD but have mutations in PARK2, 6 and 7 with *in vivo* ^{18}F-dopa PET findings and pathology unlike that found in Lewy body PD (Pramstaller et al., 2001; Khan et al, 2002). In addition, if the Alu element in *DCP1/ACE* is not the true variant but is in absolute linkage disequilibrium with another sequence either within *ACE* itself or in another gene close by this will in itself lead to discrepancies between different cohorts studied (Kehoe et al., 2003).

The *DCP1/ACE* insertion/deletion polymorphism has been studied in two other PD populations. Mellick and co-workers studied the ACE gene in an Australian PD population and found no association (Mellick et al., 1999). In a recent report in a Taiwanese population (Lin et al,, 2002) the frequency of the DD genotype was increased in the PD population (p=0.04) although the authors hypothesise that there may be an overlap of susceptibility alleles: the DD genotype is associated with both cardiovascular and cerebral vascular disease (Cambien et al., 1992; Markus et al,, 1995) and PD patients are 2.5 times more likely to develop vascular disease (Markus et al., 1995). However, the frequencies of DD, DI and II genotypes in Taiwanese controls (12, 45, 43% respectively) was markedly different from both ours (30.3, 49.7 and 20.0 percent respectively) and other reports (Linpaintner et al., 1995). This also highlights the variation in allele frequencies amongst different racial groups.

Alternatively population stratification, statistical artefact, small sample sizes and poorly matched controls may be contributing to a false positive association in our data. However the control groups (unlike the two diseased cohorts) were in Hardy Weinberg equilibrium with allele frequencies identical to a control population published with 2340 controls (Lindpaintner et al., 1995). Replication of the association in two PD cohorts showed a strongest association with pathologically proven cases. Moreover the gene product-disease phenomenon is a feasible one.

Even larger controlled studies with of the order of 1,000 cases (preferably histopathologically proven) with as many controls using a dense linkage disequilibrium map of single nucleotide polymorphisms will be necessary to confirm these findings.

Presently there are no studies of the presence or activity of ACE protein in PD brains; this will be crucial in definitively resolving its contribution to the pathogenesis of PD.

7. SUMMARY OF THIS THESIS

This thesis was undertaken during an exciting time in the history of inherited Parkinson's disease and parkinsonism; a time when the was an explosion in the identification of disease-causing loci.

Familial parkinsonism encompasses a heterogeneous group of diseases. Familial 'parkinsonism' was observed in early onset, recessive disease with atypical phenotypes, normal sense of smell and patterns of nigrostriatal dysfunction and rate of progression of functional imaging unlike that seen in idiopathic PD. These features will assist in distinguishing the commomest form of early onset parkinsonism, 'parkin disease' from idiopathic PD.

Functional imaging in asymptomatic heterozygotes implied that parkin and PINK1 proteins exhibited the phenomenon of haploinsufficiency. Currently the exact implication of carrying one mutant parkin allele in asymptomatic subjects is undefined however prospective studies of clinical features and serial functional imaging of larger cohorts are required; parkin heterozygosity could be used as a biomarker of presymptomatic disease.

Familial Parkinson's disease with a typical phenotype, pathology and pattern of ^{18}F-dopa uptake similar to PD was observed in an autosomal dominant British kindred. Linkage analysis was used to map the disease to a 50cM region on the short arm of chromosome 12. This confirmed locus heterogeneity in autosomal dominant Parkinson's disease by overlapping a locus, PARK8, that had just been reported in a Japanese kindred, with an identical phenotype. Subsequent work by colleagues identified mutations in LRRK2 which has since been shown to be the commonest cause of ADPD world-wide.

8. REFERENCES

Aarsland D, Larsen JP, Lim NG et al. Range of neuropsychiatric disturbances in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 1999; 67: 492-6.

Aasly JO, Toft M, Fernandez-Mata I et al. Clinical features of LRRK2-associated Parkinson's disease in central Norway. Ann Neurol. 2005 May; 57(5):762-5.

Abbas N, Lucking CB, Ricard S et al. A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. Hum Mol Genet 1999; 8: 567-574.

Abou-Sleiman P.M, Healy D.G, Quinn N et al. The role of pathogenic DJ-1 mutations in Parkinson's disease. Ann Neurol 2003; 54: 283-6.

Adams JR, van Netten H, Schulzer Met al. PET in LRRK2 mutations: comparison to sporadic Parkinson's disease and evidence for presymptomatic compensation. Brain. 2005 Dec;128(Pt 12):2777-85.

Agid Y. Parkinson's disease: pathophysiology. Lancet. 1991 Jun 1;337(8753):1321-4.

Allen AM, MacGregor DP, Chai SY et al. Angiotensin II receptor binding associated with nigrostriatal dopaminergic neurons in human basal ganglia. Ann Neurol. 1992 Sep;32(3):339-44.

Athanassiadou A, Voutsinas G, Psiouri L et al. Genetic analysis of families with Parkinson disease that carry the Ala53Thr mutation in the gene encoding alpha-synuclein. Am J Hum Genet. 1999 Aug;65(2):555-8.

Bandopadhyay R, Kingsbury AE, Cookson MR et al. The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinsons disease. Brain 2004; 27: 420–430.

Bennett P, Nicholl DJ. Absence of the G209A mutation in the alpha-synuclein gene in British families with Parkinson's disease. Neurology. 1998 Apr;50(4):1183.

Ben-Shlomo Y, Marmot MG. Survival and cause of death in a cohort of patients with parkinsonism: possible clues to aetiology? J Neurol Neurosurg Psychiatry. 1995 Mar; 58(3):293-9.

Bentivoglio AR, Cortelli P, Valente EM, et al. Phenotypic characterisation of autosomal recessive PARK6-linked parkinsonism in three unrelated Italian families. Mov Disord. 2001; 16 : 999-1006.

Bernheimer H, Birkmayer W, Hornykiewicz O et al.Clinical, morphological and neurochemical correlations. J Neurol Sci. 1973 Dec; 20: 415-55.

Bezard E. and Gross C.E. Compensatory mechanisms in experimental and human parkinsonism: Towards a dynamic approach. Prog. Neurobiol 1998; 55: 93-116.

Bird T, Jarvik G, Wood NW. Genetic Association studies. Genes in search of disease. Neurology 2001; 57: 1153-1154.

Bonifati V, Rizzu P, Van Baren et al. Mutations in the DJ-1 gene is associated with autosomal recessive early-onset parkinsonism. Science 2003 299:256-259.

Booij J, Tissingh G, Winogrodzka A et al. Imaging of the dopaminergic neurotransmission system using single-photon emission tomography and positron emission tomography in patients with parkinsonism. Eur J Nucl Med. 1999 Feb;26(2):171-82.

Brooks DJ, Ibanez V, Sawle GV, et al. Differing patterns of striatal [18F]-dopa uptake in Parkinson's disease, Multiple system atrophy and Progressive Supranuclear palsy. Ann Neurol. 1990; 28:547-555.

Broussolle E, Lucking CB, Ginovart N, et al. [18F]-dopa PET study in patients with juvenile-onset PD and *parkin* gene mutations. Neurology 2000; 26:877-879.

Calne DB, Langston JW, Martin W et al. Positron emission tomography after MPTP: observations relating to the cause of Parkinson's disease. Nature. 1985 Sep 19-25; 317(6034):246-8.

Calne DB, Lees AJ. Late progression of post-encephalitic Parkinson's syndrome. Can J Neurol Sci. 1988; 15(2): 135-8.

Cambien F, Poirier O, Lecerf L et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature. 1992 Oct 15; 359(6396): 641-4.

Canet-Aviles RM, Wilson MA, Miller DW et al. The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization. Proc Natl Acad Sci U S A. 2004; 101: 9103-8.

Chartier-Harlin MC, Kachergus J, Roumier C et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet. 2004 Sep 25-Oct 1;364(9440):1167-9.

Chiano M, Clayton D. Fine Genetic mapping using haplotype analysis and the missing data problem. Ann Hum Genet 1998;62:55-60.

Clayton D,George J. Synucleins in synaptic plasticity and neurodegenerative disorders. J Neurosci Res 1999; 58:120-129.

Collins FS, Patrinos A, Jordan E, Chakravarti A et al. New goals for the U.S. Human Genome Project: 1998-2003. Science. 1998 23; 282(5389):682-9.

Daniel S, Hawkes C. Preliminary diagnosis of Parkinsons' disease using olfactory bulb pathology. Lancet 1992; 340: 186.

De Michele G, Filla A, Volpe G et al. Environmental and genetic risk factors in Parkinson's disease: a case-control study in southern Italy. Mov Disord. 1996 Jan;11(1):17-23.

De Rijk M, Tzourio C, Breteler M et al. Prevalence of Parkinsonism and Parkinson's disease in Europe. J Neurol Neuropsychiatry 1997; 62:10-15.

Dekker MCJ, Bonifati V, van Duijn CM. Parkinson's disease: piecing together a genetic jigsaw. Brain 2003; 126:1722-33.

Dekker MC, Galjaard RJ, Snijders PJ et al. Brachydactyly and short stature in a kindred with early-onset parkinsonism. Am J Med Genet A. 2004 Sep 15; 130(1):102-4.

Dekker MC, Eshuis SA, Maguire RP et al. PET neuroimaging and mutations in the DJ-1 gene. J Neural Transm 2004a Dec; 111(12):1575-81.

Doty R, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardised microencapsulated test of olfactory function. Physiology and Behaviour 1984; 32: 489-502.

Doty R, Deems D, Stellar S. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurological signs, disease stage or disease duration. Neurology 1988; 38: 1237-1244.

Doty RL, Perl DP, Steele J et al. The odour identification deficit of Guam parkinson-dementia: equivalence to that of Alzheimer's and idiopathic Parkinsons' disease. Neurology 1991; 41: 77-80.

Doty R, Singh A, Tetrud J et al. Lack of major olfactory dysfunction in MPTP-induced parkinsonsim. Ann Neurol 1992; 32: 87-100.

Duda JE, Giasson BI, Chen Q et al. Widespread nitration of pathological inclusions in neurodegenerative synucleinopathies. Am J Pathol. 2000 Nov; 157(5):1439-45.

Dwoskin LP, Jewell AL, Cassis LA. DuP 753, a nonpeptide angiotensin II-1 receptor antagonist, alters dopaminergic function in rat striatum. Naunyn Schmiedebergs Arch Pharmacol. 1992 Feb; 345(2): 153-9.

Eaves IA et al. The genetically isolated populations of Finland and Sardinia may not be a panacea for linkage disequilibrium mapping of common disease genes. Nat Genet. 2000; 25(3):320-3.

Eidelberg D, Takikawa S, Dhawan V, et al. Striatal [18F]-dopa uptake: absence of an ageing effect. J Cereb Blood Flow Metab. 1993; 13:881-888.

Engelender S, Kaminsky Z, Guo X et al. Synphilin-1 associates with alpha-synuclein and promotes the formation of cytosolic inclusions. Nat. Genet. 1999; 22: 110–114.

Eriksen JL, Dawson TM, Dickson DW et al. Caught in the act: α-synuclein is the culprit in Parkinson's disease. Neuron. 2003; 4: 453-6.

Fahn S, Elton R, Members of the UPDRS Development Committee. Fahn S, Marsden CD, Calne DB, et al. Recent developments in Parkinson's disease, Vol 2. Florham Park, NJ. Macmillan Health Care Information 1987 pp153-163, 293-304.

Farrer M, Wavrant-De Vrieze F, Crook R et al. Low frequency of alpha-synuclein mutations in familial Parkinson's disease. Ann Neurol. 1998 Mar;43(3):394-7.

Farrer M, Chan P, Chen R et al. Lewy bodies and parkinsonism in families with Parkin mutations. Ann Neurol 2001; 50: 293-300.

Feany MB, Bender WW. A Drosophila model of Parkinson's disease. Nature. 2000; 404: 394-8.

Firnau G., S. Sood, R. Chirakal, C. Nahmias, E. S. Garnett. Cerebral metabolism of 6-18F-Fluoro-L-3,4-Dihydroxyphenyalanine in the primate. J Neurochem 48 1987 1077-1082.

Folstein MF, Folstein SE, McHugh PR. J Psychiatr Res 1975; 12: 189-198.

Friston K, Ashburner J, Poline JB, et al. Spatial registration and normalization of images. Hum Brain Mapp 1995; 2:165-189).

Funayama M, Hasegawa K, Kowa H et al. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurology 2002; 51:296-301.

Gambaro G, Anglani F, D'Angelo A. Association studies of genetic polymorphisms and complex disease. Lancet 2000; 355:308-311.

Garnett ES, Firnau G, Nahmias C. Dopamine visualized in the basal ganglia of living man. Nature. 1983 Sep 8-14; 305(5930):137-8.

Gasser T, Muller-Myhsok B, Wszolek ZK et al. A susceptibility locus for Parkinsons' disease maps to chromosome 2p13. Nat Genet 1998; 18:262-265.

Giasson BI, Lee VM. Parkin and the molecular pathways of Parkinson's disease. Neuron. 2001 Sep 27; 31(6):885-8.

Gibb W, Lees A. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988; 51: 745-52.

Golbe LI, Di Iorio G, Bonavita Vet al. A large kindred with autosomal dominant Parkinson's disease. Ann Neurol. 1990 Mar; 27(3):276-82.

Gowers, W. R. A Manual of Diseases of the Nervous System. Vol. I. Diseases of the nerves and spinal cord. Philadelphia: P. Blakiston's Son & Co. (3rd ed.) 1900.

Guttman M, Yong VW, Kim Su et al. Asymptomatic striatal dopamine depletion: PET scans in unilateral MPTP monkeys. Synapse. 1988; 2(5):469-73.

Guttman M. Receptors in the basal ganglia. Can J Neurol Sci. 1987 Aug; 14(3 Suppl):395-401. Review.

Harhangi BS, Farrer MJ, Lincoln S et al. The Ile93Met mutation in the ubiquitin carboxy-terminal-hydrolase-L1 gene is not observed in European cases with familial Parkinson's disease. Neurosci Lett. 1999 Jul 23; 270(1):1-4.

Hayashi S, Wakabayashi K, Ishikawa A et al. An autopsy case of autosomal recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene. Mov Dis 2000; 15: 884-888.

Hering R, Petrovic S, Mietz EM et al. Extended mutation analysis and association studies of Nurr1 (NR4A2) in Parkinson disease. Neurology. 2004; 62:1231-2.

Hernandez DG, Paisan-Ruiz C, McInerney-Leo A et al. Clinical and positron emission tomography of Parkinson's disease caused by LRRK2.Ann Neurol. 2005 Mar; 57(3):453-6.

Hilker R, Klein C, Ghaemi M et al. Positron emission tomographic analysis of the nigrostriatal dopaminergic system in familial parkinsonism associated with mutations in the Parkin gene. Ann Neurol 2001; 49: 367-376.

Hoehn M, Yahr M. Parkinsonism: onset, progression and mortality. Neurology 1967; 17:427-42.

Hu J, Igarashi A, Kamata M et al. Angiotensin-converting enzyme degrades Alzheimer amyloid beta-peptide (A beta); retards A beta aggregation, deposition, fibril formation; and inhibits cytotoxicity. J Biol Chem. 2001 Dec 21; 276(51):47863-8.

Hubble JP, Cao T, Hassanein RE et al. Risk factors for Parkinson's disease. Neurology 1993 Sep; 43(9):1693-7.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of Clinical Diagnosis of idiopathic Parkinson's disease: a clinicopathologic study of 100 cases. J Neurol Neurosurg Psychiatry 1992:55:181-184.

Huynh D, Scoles D, Ho T et al. Parkin is associated with actin filaments in neuronal and nonneuronal cells. Ann Neurol 2000; 48: 737-44.

Ibanez P, Bonnet AM, Debarges B et al. Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. Lancet. 2004 Sep 25-Oct 1; 364(9440):1169-71.

Jarman PR, Bandmann O, Marsden CD, et al. GTP cyclohydrolase I mutations in patients with dystonia responsive to anticholinergic drugs. J Neurol Neurosurg Psychiatry 1997; 63: 304-8.

Johnson J, Hague SM, Hanson M et al. SNCA multiplication is not a common cause of Parkinson disease or dementia with Lewy bodies. Neurology. 2004 Aug 10; 63(3):554-6.

Kachergus J, Mata IF, Hulihan M et al. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. Am J Hum Genet. 2005 Apr; 76(4): 672-80.

Kann M, Jacobs H, Mohrmann K, et al. Role of *Parkin* mutations in 111 community based patients with early – onset parkinsonism. Ann Neurol 2002; 51: 621-5.

Kay DM, Kramer P, Higgins D et al. Escaping Parkinson's disease: A neurologically healthy octogenarian with the LRRK2 G2019S mutation. Movement Disorders. 2005 Aug; 20(8):1077-1078.

Keavney B, McKenzie CA, Connell JM et al. Measured haplotype analysis of the angiotensin-I converting enzyme gene. Hum Mol Genet. 1998 Oct; 7(11):1745-51.

Kehoe PG, Katzov H, Feuk L, et al. Haplotypes extending across ACE are associated with Alzheimer's disease. Hum Mol Genet. 2003 Apr 15; 12(8):859-67.

Kehoe PG, Russ C, McIlory S et al. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease.Nat Genet. 1999 Jan;21(1):71-2.

Khan NL, Valente E, Bentivoglio A et al. Clinical and subclinical dopaminergic dysfunction in PARK6-linked parkinsonism: an ^{18}F-Dopa PET study. Ann Neurol 2002; 52:849-853.

Khan N, Brooks D, Parvese N, et al. Progression of nigrostriatal dysfunction in a parkin kindred: an ^{18}F-dopa PET and clinical study. Brain 2002; 125:2248-2256.

Khan NL, Graham E, Critchley P et al. Parkin disease: a phenotypic study of a large case series. Brain 2003; 126:1279-1292.

Khan NL, Katzenschlager R, Watt H et al. Olfaction differentiates parkin disease from early-onset parkinsonism and Parkinson's disease. Neurology 2004 Apr 13;62(7):1224-6.

Khan NL, Scherfler C, Graham E et al. Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single parkin mutation: an ^{18}F-dopa PET study. Neurology 2005 Jan 11;64(1):134-6.

Khan NL, Jain S, J Holton et al. Mutations in the gene (LRRK2) encoding dardarin (PARK8) causing familial Parkinson's disease in a British kindred; clinical, pathological, olfactory and functional imaging data. Brain 2005 Dec;128:2786-96.

Kish SJ, Shannak K, Hornykiewicz O. Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med. 1988 Apr 7; 318(14):876-80.

Kitada T, Asakawa S, Hatorri N et al. Mutations in the Parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392:605-608.

Kitada T, Asakawa S, Hatorri N et al. Mutations in the Parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998; 392:605-608.

Klein C, Pramstaller PP, Berhard K, et al. Parkin deletions in a family with adult onset, tremor dominant parkinsonism: expanding the phenotype. Ann Neurol 2000; 48: 65-71.

Koller WC, Langston JW, Hubble JP et al. Does a long preclinical period occur in Parkinson's disease? Geriatrics. 1991 Aug;46 Suppl 1:8-15. Review.

Kruger R, Kuhn W, Muller T et al. Ala30Pro mutation in the gene encoding a-synuclein in Parkinson's disease. Nat Genet 1998: 18:106-108.

Kruger R, Menezes A, Saecker V, et al. Increased susceptibility to Sporadic Parkinson's disease by a certain combined a-synuclein/apolipoprotein E genotype. Ann Neurol 1999:45;5;1-7.

Kuroda Y, Mitsui T, Akaike M et al. Homozygous deletion mutation of the parkin gene in patients with atypical parkinsonsim. J Neurol Neurosurg Psychiatry 2000; 71: 231-234.

Langston JW, Koller WC. Preclinical detection of Parkinson's disease. The next frontier: presymptomatic detection. Geriatrics. 1991 Aug; 46 Suppl 1:5-7.

Lathrop GM, Lalouel JM, Julier C et al. Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 1985; 37:482-498.

Lathrop GM and Lalouel JM. Efficient computations in multilocus linkage analysis. Am J Hum Genet 1988; 42:498-505.

Lazzarini AM, Myers RH, Zimmerman TR et al. A clinical genetic study of Parkinson's disease: evidence for dominant transmission. Neurology 1994 Mar;44(3 Pt 1):499-506.

Le WD, Xu P, Jankovic J et al. Mutations in NR4A2 associated with familial Parkinson disease. Nat Genet. 2003 Jan; 33(1):85-9.

Leenders KL, Poewe WH, Palmer AJ et al. Inhibition of L-18F] fluorodopa uptake into human brain by amino acids demonstrated by positron emission tomography. Ann Neurol. 1986 Aug; 20(2): 258-62.

Leenders KL, Salmon EP, Tyrrell P et al.The nigrostriatal dopaminergic system assessed in vivo by positron emission tomography in healthy volunteer subjects and patients with Parkinson's disease. Arch Neurol. 1990 Dec; 47(12):1290-8.

Leroy E, Boyer R, Auburger G et al. The ubiquitin pathway in Parkinson's disease. Nature. 1998 Oct 1; 395(6701):451-2.

Liberini P, Parola P, Spano PF et al. Olfactory dysfunction in dementia associated with Lewy bodies. Parkinsonism Rel Disord 1999; 5: 30.

Lin JJ, Yueh KC, Chang DC, Lin SZ. Association between genetic polymorphism of angiotensin-converting enzyme gene and Parkinson's disease. J Neurol Sci. 2002 Jul 15; 199(1-2):25-9.

Lincoln S, Vaughan J, Wood N et al. Low frequency of pathogenic mutations in the ubiquitin carboxy-terminal hydrolase gene in familial Parkinson's disease. Neuroreport. 1999 Feb 5; 10(2):427-9.

Lindholm E, Ekholm B, Shaw S et al. A schizophrenia-susceptibility locus at 6q25, in one of the world's largest reported pedigrees. Am J Hum Genet 2001; 69: 96-105.

Lindpaintner K, Pfeffer MA, Kreutz R et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med. 1995 Mar 16; 332(11): 706-11.

Liolitsa D, Powell J, Lovestone S. Genetic variability in the insulin signalling pathway may contribute to the risk of late onset Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2002 Sep; 73(3):261-6.

Lowe J, McDermott H, Landon M et al. Ubiquitin carboxyl-terminal hydrolase (PGP 9.5) is selectively present in ubiquitinated inclusion bodies characteristic of human neurodegenerative diseases. J Pathol. 1990 Jun; 161(2):153-60.

Lucking C, Bonifati V, Periquet M et al. Pseudo-dominant inheritance and exon 2 triplication in a family with parkin gene mutations. Neurology 2001; 57: 924-927.

Lucking C, Durr A, Bonifiati V et al. Association between early onset Parkinson's disease and mutations in the parkin gene. N Engl J Med 2000; 342: 1560-1567.

MacDonald Critchley. Sir William Gower, 1845-1915. A Biographical Appreciation.

Marder K, Tang MX, Mejia H et al. Risk of Parkinson's disease among first-degree relatives: A community-based study. Neurology. 1996 Jul;47(1):155-60.

Maruyama M, Ikeuchi T, Saito M, et al. Novel mutations, pseudo-dominant inheritance, and possible familial effects in patients with autosomal recessive juvenile parkinsonism. Ann Neurol 2000; 48: 245-50.

Marx FP, Holzmann C, Strauss KM et al.Identification and functional characterization of a novel R621C mutation in the *synphilin-1* gene in Parkinson's disease. Hum Mol Genet. 2003; 12: 1223-31.

Masliah E, Rockenstein E, Sun Y et al. Dopaminergic cell loss and inclusion body formation in alpha-synuclein mice: implication in neurodegenerative disorders. Science 2000; 287: 1265-1269.

Mata IF, Taylor JP, Kachergus J et al. LRRK2 R1441G in Spanish patients with Parkinson's disease. Neurosci Lett. 2005 Jul; 382(3):309-11.

McKeith IG, Galasko D, Kosaka K et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996; 47(5):1113-1124.

McKenzie CA, Abecasis GR, Keavney B et al. Trans-ethnic fine mapping of a quantitative trait locus for circulating angiotensin I-converting enzyme (ACE). Hum Mol Genet. 2001 May 1; 10(10): 1077-84.

McKenzie CA, Julier C, Forrester T et al. Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: evidence for two quantitative-trait loci. Am J Hum Genet. 1995 Dec; 57(6): 1426-35. Erratum in: Am J Hum Genet 1996 Mar; 58(3):648.

Mellick GD, Buchanan DD, McCann SJ et al. The ACE deletion polymorphism is not associated with Parkinson's disease. Eur Neurol. 1999;41(2):103-6.

Mori H, Kondo T, Yokochi M et al. Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. Neurology 1998; 51: 890-892.

Morton NE. Sequential tests for the detection of linkage. Am J Hum Genet 1955;7:277-318.

Morrish PK, Sawle GV, DJ Brooks. An [18F]-dopa PET and clinical study of the rate of progression in Parkinson's disease. Brain 1996; 119:585-591.

Morrish PK, Rakshi JS, Bailey DL et al. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. J Neurol Neurosurg Psychiatry. 1998 Mar; 64(3):314-9.

Muenter MD, Forno LS, Hornykiewicz O et al. Hereditary form of parkinsonism-dementia. Ann Neurol 1998; 43:768-81.

Munoz E, Pastor P, Marti M, et al. A new mutation in the parkin gene in a patient with a typical autosomal recessive juvenile parkinsonism. Neurosci Lett 2000; 289: 66-68.

Nicholl D, Vaughan J, Khan NL et al. Two large British kindreds with familial Parkinson's disease: a clinico-pathological and genetic study. Brain 2002; 125:44-57.

Nichols WC, Uniacke SK, Pankratz N et al. Evaluation of the role of Nurrl in a large sample of familial Parkinson's disease. Mov Disord. 2004; 19: 649-55.

Nussbaum RL. Putting the parkin into Parkinson's. Nature. 1998 Apr 9;392(6676):544-5.

Obata T, Yamanaka Y. Parkinsonism induced by MPTP and free radical generation. Nippon Yakurigaku Zasshi. 2001 Feb;117(2):105-10. Review. Japanese.

Obata T. Dopamine efflux by MPTP and hydroxyl radical generation. J Neural Transm. 2002 Sep; 109(9):1159-80. Review.

Oertel WH, Hoglinger GU, Caraceni T et al. Depression in Parkinson's disease. An update. Adv Neurol. 2001; 86: 373-83.

Oliveira SA, Scott WK, Martin ER, et al. Parkin mutations and susceptibility alleles in late-onset Parkinson's disease. Ann Neurol. 2003; 53: 624-629.

Paisan-Ruiz C, Jain S, Whitney Evans E et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004 Nov 18; 44(4):595-600.

Pan TC, Zhang RZ, Sudano DG et al. New molecular mechanism for Ullrich congenital muscular dystrophy: a heterozygous in-frame deletion in the COL6A1 gene causes a severe phenotype. Am J Hum Genet. 2003 Aug; 73(2):355-69.

Papadimitriou A, Veletza V, Hadjigeorgiou GM et al. Mutated alpha-synuclein gene in two Greek kindreds with familial PD: incomplete penetrance? Neurology. 1999 Feb;52(3):651-4.

Parkinson J. An Essay on the Shaking Palsy. London, Whittingham & Rowland, 1817. Reprinted in Medical Classic, 1938, 2: 946-97.

Pate BD, Kawamata T, Yamada T et al Correlation of striatal fluorodopa uptake in the MPTP monkey with dopaminergic indices. Ann Neurol. 1993 Sep;34(3):331-8.

Payami H, Larsen K, Bernard S et al. Increased risk of Parkinson's disease in parents and siblings of patients. Ann Neurol. 1994 Oct;36(4):659-61.

Payami H, Zareparsi S, James D et al. Familial aggregation of Parkinson disease: a comparative study of early-onset and late-onset disease. Arch Neurol. 2002 May;59(5):848-50.

Pearce R, Hawkes C, Daniel S. The anterior olfactory nucleus in Parkinson's disease. Move Disord 1995; 10: 283-287.

Periquet M, Lucking CB, Vaughan JR et al.. Origin of mutations in the parkin gene in Europe: exon rearrangements are independents whereas point mutations may result from founder effects. Am J Hum Genet 2001: 68: 617-626.

Piccini P, Burn DJ, Ceravolo R et al. The role of inheritance in sporadic Parkinson's disease: evidence from a longitudinal study of dopaminergic function in twins. Ann Neurol 1999 May; 45(5):577-82.

Piccini P, Morrish PK, Turjanski N et al. Dopaminergic function in familial Parkinson's disease: a clinical and 18F-dopa positron emission tomography study. Ann Neurol. 1997; 41: 222-9.

Piccini P, Turjanski N, Brooks DJ. PET studies of the striatal dopaminergic system in Parkinson's disease (PD). J Neural Transm 45 (Suppl) (1995) 123-131.

Polymeropoulos MH, Higgins JJ, Golbe LI et al. Mapping of the gene for Parkinson's disease to chromosome 4q12-q23. Science 1996; 274:2045-2047.

Polymeropoulos MH, Lavedan C, Leroy E et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997 276(5321):2045-7.

Portman AT, Giladi N, Leenders KL et al. The nigrostriatal dopaminergic system in familial early onset parkinsonism with parkin mutations. Neurology. 2001; 56:1759-1762.

Quinn N, Critchley P, Marsden CD. Young onset Parkinson's disease. Mov Disord 1987; 2: 73-91.

Rakshi JS, Uema T, Ito K, Bailey DL et al. Frontal, midbrain and striatal dopaminergic function in early and advanced Parkinson's disease A 3D [(18)F]dopa-PET study. Brain. 1999; 122: 1637-50.

Reardon KA, Mendelsohn FA, Chai SY et al. The angiotensin converting enzyme (ACE) inhibitor, perindopril, modifies the clinical features of Parkinson's disease. Aust N Z J Med. 2000 Feb;30(1):48-53.

Rieder MJ, Taylor SL, Clark AG, Nickerson DA. Sequence variation in the human angiotensin converting enzyme. Nat Genet. 1999 May; 22(1): 59-62.

Rigat B, Hubert C, Corvol P et al. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res. 1992 Mar 25; 20(6): 1433.

Ros R, Gomez Garre P, Hirano Met al. Genetic linkage of autosomal dominant progressive supranuclear palsy to 1q31.1. Ann Neurol. 2005 May;57(5):634-41.

Salminen O, Seppa T, Gaddnas H et al. The effects of acute nicotine on the metabolism of dopamine and the expression of Fos protein in striatal and limbic brain areas of rats during chronic nicotine infusion and its withdrawal. J Neurosci. 1999 Sep 15;19(18):8145-51.

Samii A, Markopoulou K, Wszolek ZK et al. PET studies of parkinsonism associated with mutation in the alpha-synuclein gene. Neurology 1999 Dec 10;53(9):2097-102.

Sawle GV, Colebatch JG, Shah A et al. Striatal function in normal ageing: implications for Parkinson's disease. Ann Neurol. 1990; 28:799-804.

Sawle GV, Leenders KL, Brooks DJ, et al. Dopa-responsive dystonia: [18F]Dopa positron emission tomography. Ann Neurol 1991; 30: 24-30.

Sawle GV, Wroe SJ, Lees AJ, et al. The Identification of Presymptomatic Parkinsonism: Clinical and [18F]-dopa Positron Emission Tomography studies in Irish Kindred. Ann Neurol 1992; 32:609-617.

Scherfler C, Khan NL, Pavese N et al. Striatal and cortical pre- and post synaptic dopaminergic dysfunction in sporadic parkin-linked parkinsonism: an 18F Dopa 11C raclopride PET study. Brain 2004 Jun; 127(Pt 6):1332-42.

Schrag A, Brown R, Ben-Shlomo Y, Marsden CD, Quinn N. Young onset Parkinson's disease revisited. Clinical features, natural history and mortality. Mov Disord 1998; 13: 885-94.

Schrag A, Jahanshahi M, Quinn NP. What contributes to depression in Parkinson's disease? Psychol Med. 2001 Jan; 31: 65-73.

Sham P, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 1995; 59;97-105.

Shimura H, Hattori N, Kubo S, et al. Familial Parkinson's disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 2000; 25:302-305.

Simonnet G, Giorguieff-Chesselet MF. Stimulating effect of angiotensin II on the spontaneous release of newly synthetized [3H] dopamine in rat striatal slices. Neurosci Lett. 1979 Dec; 15(2-3): 153-8.

Singleton A, Gwinn-Hardy K, Sharabi Y et al. Association between cardiac denervation and parkinsonism caused by alpha-synuclein gene triplication. Brain. 2004 Apr; 127(Pt 4):768-72.

Singleton AB, Farrer M, Johnson J et al. *α–Synuclein* locus triplication causes Parkinson's disease. Science. 2003; 302: 841.

Singleton N, Bumpstead R, O'Brien M, Lee A, Meltzer H. Psychiatric morbidity among adults living in private households. London: The Stationery Office; 2002.

Snow BJ, Peppard RF, Guttman Met al. Positron emission tomographic scanning demonstrates a presynaptic dopaminergic lesion in Lytico-Bodig. The amyotrophic lateral sclerosis-parkinsonism-dementia complex of Guam. Arch Neurol. 1990 Aug;47(8):870-4.

Sobel E, Lang K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. Am J Hum Genet 2002; 58: 1323-1337.

Solano S, Miller D, Augood S et al. Expression of alpha synuclein, parkin and ubiquitin carboxy-terminal hydrolase L1 mRNA in human brain: genes associated with familial Parkinson's disease. Ann Neurol 2000; 47: 201-210.

Solano SM, Miller DW, Augood SJ et al. Expression of alpha-synuclein, parkin, and ubiquitin carboxy-terminal hydrolase L1 mRNA in human brain: genes associated with familial Parkinson's disease. Ann Neurol. 2000 Feb; 47(2):201-10.

Spillantini MG, Schmidt ML, Lee VM et al. A-Synuclein in Lewy bodies. Nature 1997; 388:839-840.

Strachan T, Read A. Human Molecular Genetics, Oxford:BIOS Scientific 1999; 286-287.

Tan EK, Khajavi M, Thornby JI et al.Variability and validity of polymorphism association studies in Parkinson's disease. Neurology 2000 Aug 22; 55(4): 533-8.

Tanner CM, Ottman R, Goldman SM et al. Parkinson disease in twins: an etiologic study. JAMA. 1999 Jan 27; 281(4):341-6.

Taillon-Miller P et al. Juxtaposed regions of extensive and minimal linkage disequilibrium in human Xq25 and Xq28. Nat Genet. 2000 Jul; 25:324-8.

Talairach J, Tournoux P. Co-planar stereotaxic atlas of the human brain. Stuttgart : Thieme; 1988.

Tani M Angiotensin converting enzyme in human brain discrete localization and biochemical properties. Jpn Heart J. 1991 Mar; 32(2):189-201.

Tassin J, Durr A, Bonnet AM et al. Levodopa responsive dystonia. GTP cyclohydrolase I or parkin mutations? Brain 2000; 123: 1112-21.

Tassin J, Durr A, Broucker T et al. Chromosome 6-linked autosomal recessive early-onset parkinsonism: Linkage in European and Algerian families, extension of the clinical spectrum, and evidence of a small homozygous deletion in one family. Am J Hum Genet 1998; 63: 88-94.

Tiret L, Kee F, Poirier O et al. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. Lancet. 1993 Apr 17; 341(8851): 991-2.

Valente E, Bentivoglio A, Dixon P, et al. Localisation of a novel locus for autosomal recessive early onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 2001: 68: 895-900.

Valente EM, Abou-Sleiman PM, Caputo V et al. Hereditary early onset Parkinson's disease is caused by mutations in *PINK1.* Science 2004; 304: 1158-60.

Valente EM, Brancati F, Ferraris A, et al. PARK6-linked parkinsonism occurs in several European families. Ann Neurol. 2002; 51: 14-8.

Van de Warrenburg BP, Lammens M, Lucking CB, et al. Clinical and pathologic abnormalities in a family with parkinsonism and parkin gene mutations. Neurology 2001; 56: 555-557.

Van Duijn C, Dekker C, Bonifati V et al,. PARK7, a novel locus for autosomal recessive early onset parkinsonism on chromosome 1p36. Am J Hum Genet 2001; 69: 629-34.

Vaughan JR, Farrer MJ, Wszolek ZK et al. Sequencing of the alpha-synuclein gene in a large series of cases of familial Parkinson's disease fails to reveal any further mutations. The European Consortium on Genetic Susceptibility in Parkinson's Disease (GSPD). Hum Mol Genet. 1998a Apr; 7(4):751-3.

Vaughan J, Durr A, Tassin J et al. The alpha-synuclein Ala53Thr mutation is not a common cause of familial Parkinson's disease: a study of 230 European cases. European

Consortium on Genetic Susceptibility in Parkinson's Disease.Ann Neurol. 1998b Aug; 44(2):270-3.

Wenham PR, Price WH, Blundell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991; 337:1158-1159.

Wenning G, Shepherd B, Hawkes C et al. Olfactory function in progressive supranuclear palsy and corticobasal degeneration. J Neurol Neurosurg Psychiatry 1995; 57: 251-2.

Wenning G, Shepherd B, Magalhaes M et al. Olfactory function in multiple system atrophy. Neurodegeneration 1993; 2: 169-171.

West A, Maraganore D, Crook J et al. Functional association of the parkin promotor with idiopathic Parkinsons' disease. Hum Mol Genet 2002;11:2787-2792.

West A, Periquet M, Lincoln S, et al. Complex relationships between parkin mutations and Parkinson's disease. Am J Med Genetics 2002; 114:584-591.

Whone AL, Moore RY, Piccini PP, Brooks DJ. Compensatory changes in the globus pallidus in early Parkinson's disease: An ^{18}F-Dopa PET Study. Neurology 2001; 56:A72.

Wintermeyer P, Kruger R, Kuhn W et al. Mutation analysis and association studies of the UCHL1 gene in German Parkinson's disease patients. Neuroreport. 2000 Jul 14;11(10):2079-82.

Xia Y, Rohan De Silva HA, Rosi BL et al. Genetic studies in Alzheimer's disease with an NACP/a-synuclein polymorphism. Ann Neurol 1996; 40:207-215.

Xia Y, Saitoh T, Kang D et al. Characterisation of the Human a-synuclein (SNCA) gene: genomic structure, transcription start site, promotor region and polymorphisms. J Alzheimers Dis. 2001 Oct; 3(5): 485-494.

Yamamura Y, Nobutaka H, Matsumine H et al. Autosomal recessive early-onset parkinsonism with diurnal fluctuation: clinicopathologic characteristics and molecular genetic identification. Brain and Dev 2000; 22: 87-91.

Yamamura Y, Sobue I, Ando K et al. Paralysis agitans of early onset with marked diurnal fluctuations of symptoms. Neurology 1973; 23: 239-244.

Yokota T, Sugawara K, Ito K et al. Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition. Biochem. Biophys. Res. Com 2003; 312: 1342–1348.

Zarranz JJ, Alegre J, Gomez-Esteban JC et al. The new mutation, E46K, of *α—synuclein* causes Parkinson and Lewy body dementia. Ann Neurol. 2004; 55:164-73.

Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. Dopamine neuron agenesis in NURR1 deficient mice. Science 1997; 276: 248-250.

Zhang Y, J Gao, Chung K et al. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of synaptic vesicle -associated protein, CDCrel-1. PNAS 2000; 97: 13354-13359.

Zetterstrom RH, Solomin L, Jansson L et al. Dopamine neuron agenesis in Nurr1-deficient mice. Science. 1997 Apr 11; 276(5310):248-50

Zimprich A, Muller-Myhsok B, Farrer M et al. The PARK8 locus in autosomal dominant parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. Am J Hum Genet. 2004 74(1): 11-9.

Zubenko GS, Marquis JK, Volicer L, Direnfeld LK, Langlais PJ, Nixon RA. Cerebrospinal fluid levels of angiotensin-converting enzyme, acetylcholinesterase, and dopamine metabolites in dementia associated with Alzheimer's disease and Parkinson's disease: a correlative study. Biol Psychiatry. 1986 Dec; 21(14):1365-81.

Zubenko GS, Volicer L, Direnfeld LK et al. Cerebrospinal fluid levels of angiotensin-converting enzyme in Alzheimer's disease, Parkinson's disease and progressive supranuclear palsy. Brain Res. 1985 Mar 4; 328(2):215-21.

9. APPENDIX

9.1 Photographs of colleagues and collaborators

Department of Molecular Neurosciences [Neurogenetics section]
Institute of Neurology Queen Square, London 2001

[photograph: in front of the National Hospital of Neurology and Neurosurgery, Queen Square, London: courtesy of the Audio-Visual Department, Institute of Neurology, London]

Back Row (left to right): Stuart, Tunde Akimbode, Catherine, Mary Sweeney, Nicholas Wood, Peter Dixon, Louise Eunson, Mike Hanna, Nana Baoteng, Ming Jen Lee

Front Row (left to right): Asra Siddiqui, Tee Pulkes, Lisa, Nick Davies, Naheed Khan, Elizabeth Graham, Danae Liolitsa, Isabel Nelson

Dr Regina Katzenschlager, Professor Andrew Lees and Dr Rohan DeSilva of the Reta Lila Weston Institute of Neurology, Institute of Neurology, Queen Square, London

[photograph taken at Movement Disorders Society meeting, Rome Italy 2004]

Professor Niall Quinn and Professor Kailash Bhatia of the Sobell Department of Motor Neuroscience & Movement disorders, Institute of Neurology, Queen Square, London

[photograph taken at EFNS meeting, Athens, Greece 2005]

Dr Paola Piccini, Clinical Sciences Centre, Imperial College, London and Dr Naheed Khan

[photograph taken at Movement Disorders Society meeting, Rome Italy 2004]

Dr Nicola Pavese, Andrew Blythe, Hyacinth Henry, Hope McDevitt and Dr Poala Piccini of the Clinical Sciences Centre, MRC Cylcotron Unit, Imperial College, London 2006

[photograph taken at CSC MRC Cyclotron unit, Hammersmith Hospital, London 2006]

9.2 Articles in the Media

THE TIMES WEDNESDAY SEPTEMBER 9 1998

Shepherds clue to Parkinson's

By Ian Murray, Medical Correspondent

RESEARCHERS are trying to trace descendants of a 17th-century family of shepherds who may have inherited a gene that makes them prone to develop Parkinson's disease.

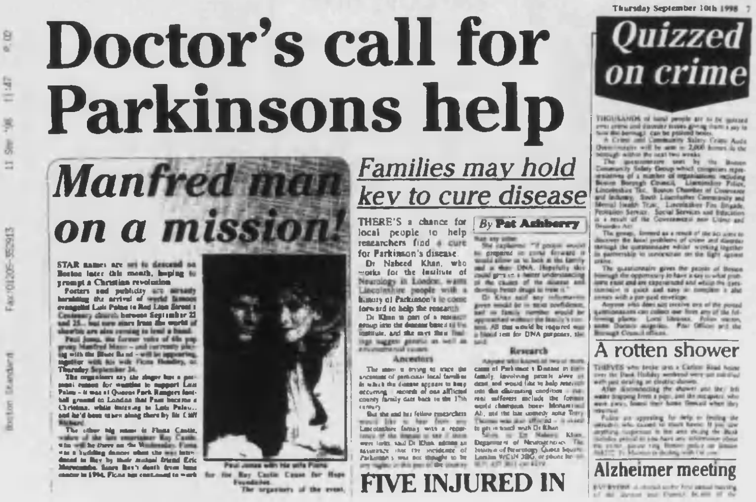
Over the past two years a team at the Institute of Neurology in London, including four neurogeneticists and two genealogists has tracked down three generations who are suffering from the disease. Although the earliest records of the family have been found in Lincolnshire, the team has traced other members in Leicestershire, Essex and Stoke-on-Trent, a number of whom suffer from the neurological disorder.

"It is rare for two or more members of the same family to have the disease so it is very helpful to find several who come from the same one," said Naheed Khan, one of the research team. She has placed an advertisement in a Lincolnshire newspaper asking for anyone with a family history of the disease to get in touch with the institute.

"The family we have identified is not responsible for Parkinson's disease, but by identifying descendants we will be able to get a better understanding of it," she said.

The Parkinson's Disease Society is sponsoring the research with a £200,000 grant.

Boston Standard, September 10th 1998



Thursday September 10th 1998 7

Doctor's call for Parkinsons help

Manfred man on a mission!

STAR names are set to descend on Boston later this month, hoping to prompt a Christian revolution.

Families may hold key to cure disease

By Pat Ashberry

THERE'S a chance for local people to help researchers find a cure for Parkinson's disease.

Dr Naheed Khan, who works for the Institute of Neurology in London, wants Lincolnshire people with a history of Parkinson's to come forward to help the research.

Ancestors

Research

FIVE INJURED IN

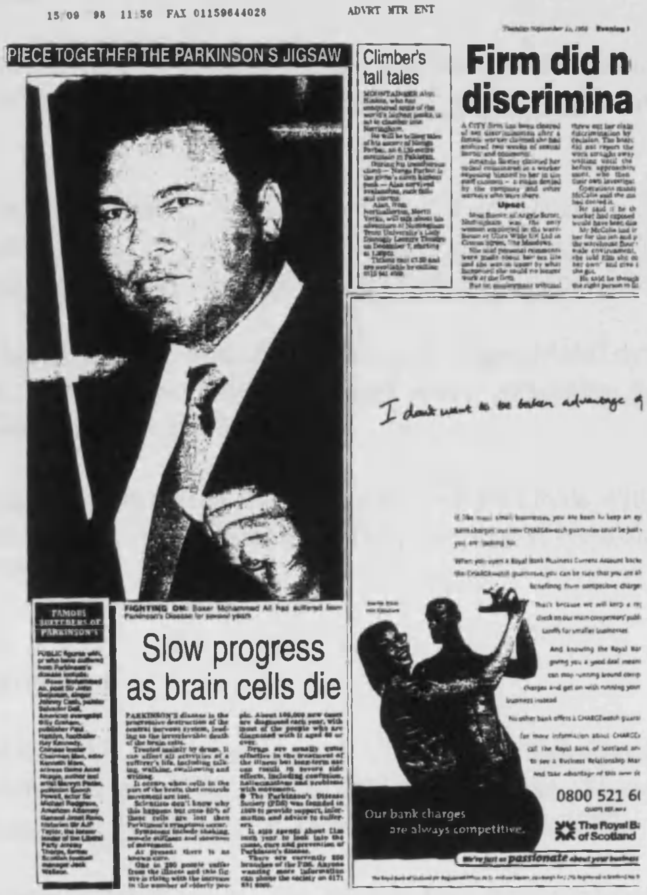
Quizzed on crime

A rotten shower

Alzheimer meeting

9.2 Articles in the Media (continued)

Evening Post, Lincoln September 1998



15/09 98 11:56 FAX 01159644028 ADVRT MTR ENT

PIECE TOGETHER THE PARKINSON'S JIGSAW

FIGHTING ON: Boxer Mohammed Ali has suffered from Parkinson's Disease for several years

FAMOUS SUFFERERS OF PARKINSON'S

Slow progress as brain cells die

Climber's tall tales

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Upset

I dont want to be taken advantage of

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9.3 Poster and platform presentations of work presented in this thesis

Poster presentations

NL Khan, Eunson L, Sweeney M et al. Evidence of Independent mutations in PARK8-linked parkinsonism. Amercian Academy of Neurology, Honolulu, Hawaii. April 2003.

NL Khan, Scherfler C, Graham E et al. Nigrostriatal dysfunction in carriers of a single mutant parkin allele. 7th International Congress of Parkinson's disease and Movement disorders. Miami Beach, Florida USA. November 2002.

NL Khan, N Parvese, D Brooks et al. Progression of Nigrostriatal dysfunction in a parkin kindred: an 18F-dopa PET and clinical study. Amercian Academy of Neurology, Philidelphia, USA. May 2001.

NL Khan, P Piccini, E Graham et al. Two large British Kindreds with Autosomal Dominant Parkinson's disease. World Congress of Neurology, London,UK June 2001.

Platform Presentations

NL Khan, EM Valente, AR Bentivigolio et al. Clinical and Subclinical Dopaminergic dysfunction in PARK6-linked parkinsonism: an 18F-dopa PET study. Amercian Academy of Neurology, Denver, USA. April 2002.

NL Khan, EA Graham, K Bhatia et al. The genetic and phenotypic variability of young onset parkinsonism. Association of British Neurologists, Oxford,UK. April 2002.

NL Khan, N Pavese, DJ Brooks et al. Progression of Nigrostriatal dysfunction in a parkin kindred: an ^{18}F-dopa PET and clinical study. Association of British Neurologists, Durham,UK. September 2001.

NL Khan, EA Graham, P Dixon et al. Parkinson's disease is not associated with the combined alpha-synuclein / apolipoprotein E susceptibility genotype. European Neurological Society, Paris, France. April 2001.

NL Khan, E Graham, J Vaughan et al.Two large British kindreds with autosomal dominant Parkinson's disease. European Neurological Society, Milan, Italy 1999.

9.4 Publications that arose from work in this thesis

Two large British Kindreds with familial Parkinson's disease: a clinicopathological and genetic study.
Nicholls D, Vaughn J, Khan NL, Ho SL, Aldous DE, Lincoln S, Farrer M, Gayton JD, Davis MB, Piccini P, Daniel SE, Lennox GG, Brooks DJ, Williams AC, Wood NW.
Brain 2002 January; 125: 1-14.

Mutations in the gene (LRRK2) encoding dardarin (PARK8) causing familial Parkinson's disease in a British kindred; clinical, pathological, olfactory and functional imaging data.
Khan NL, Jain S, J Holton, N Pavese, L Eunson, M G Sweeney, M Ganguly, W Gilks, J Vaughan, R Katzenschlager, J Gayton, G Lennox, T Revesz, A Singleton, D Nicholl, D Brook, A J Lees, P Piccini, M Davis, N W Wood.
Brain 2005 Dec; 128:2786-96.

Parkin disease a phenotypic study of a large case series.
Khan NL, Graham E, Critchley P Schrag AE, Wood N, Lees AJ, Bhatia KP, Quinn N.
Brain. 2003 June; 126: 1279-92.

Olfactory testing differentiates parkin disease from early onset parkinsonism and Parkinson's disease.
Khan NL, Katzenschlager R, Watt Bhatia KP, Wood NW, Quinn N, Lees AJ.
Neurology 2004 Apr 13; 62(7): 1224-6.

Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single parkin mutation.
Khan NL, Scherfler C, Graham E, Bhatia KP, Quinn N, Lees A J, Brooks D J, Wood N W, Piccini P.
Neurology 2005 11; 65(1):134-6

Clinical and Subclinical Dopaminergic dysfunction in autosomal recessive PARK6-linked parkinsonism: an ^{18}F-dopa PET study.
Khan NL, Valente EM, Bentivoglio A, Wood NW, Albanese A, Brooks D, Piccini P.
Annals of Neurology 2002 52; (6): 849-53.

Progression of Nigrostriatal dysfunction in a parkin kindred: an ^{18}F-dopa PET and clinical study.
Khan NL, Brooks DJ, Pavese N, Sweeney MG, Wood NW, Lees AJ, Piccini P.
Brain 2002; 125: 2248-2256.

Parkinson's disease is not associated with the combined alpha-synuclein/apolipoprotein E susceptibility genotype.
Khan N, Graham E, Dixon P, Morris C et al.
Annals of Neurology 2001 May; 49(5); 665-8.

Brain (2002), **125,** 44–57

Two large British kindreds with familial Parkinson's disease: a clinico-pathological and genetic study

D. J. Nicholl,[1,*] J. R. Vaughan,[2,*] N. L. Khan,[2] S. L. Ho,[7] D. E. W. Aldous,[1] S. Lincoln,[8] M. Farrer,[8] J. D. Gayton,[5] M. B. Davis,[2] P. Piccini,[3] S. E. Daniel,[4] G. G. Lennox,[6] D. J. Brooks,[2,3] A. C. Williams[1] and N. W. Wood[2]

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doi:10.1093/brain/awh667

Brain (2005), **128**, 2786–2796

Mutations in the gene *LRRK2* encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data

Naheed L. Khan,[1] Shushant Jain,[1,4,9] John M. Lynch,[1] Nicola Pavese,[5] Patrick Abou-Sleiman,[1] Janice L. Holton,[3] Daniel G. Healy,[1] William P. Gilks,[1] Mary G. Sweeney,[1] Milan Ganguly,[3] Vaneesha Gibbons,[1] Sonia Gandhi,[1] Jenny Vaughan,[1] Louise H. Eunson,[1] Regina Katzenschlager,[4] Juliet Gayton,[6] Graham Lennox,[7] Tamas Revesz,[3] David Nicholl,[8] Kailash P. Bhatia,[2] Niall Quinn,[2] David Brooks,[5] Andrew J. Lees,[1,4,9] Mary B. Davis,[1] Paola Piccini,[5] Andrew B. Singleton[9] and Nicholas W. Wood[1]

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DOI: 10.1093/brain/awg142 Advanced Access publication April 8, 2003 *Brain* (2003), **126,** 1279–1292

Parkin disease: a phenotypic study of a large case series

Naheed L. Khan,[1] Elizabeth Graham,[1,†] Peter Critchley,[4] Anette E Schrag,[2] Nicholas W. Wood,[1]
Andrew J. Lees,[1] Kailash P. Bhatia[2] and Niall Quinn[2]

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Brain (2002), **125**, 2248–2256

Progression of nigrostriatal dysfunction in a *parkin* kindred: an [^{18}F]dopa PET and clinical study

Naheed L. Khan,[1,2] David J. Brooks,[1] Nicola Pavese,[1] Mary G. Sweeney,[2] Nicholas W. Wood,[2]
Andrew J. Lees[3] and Paola Piccini[1]

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Olfaction differentiates parkin disease from early-onset parkinsonism and Parkinson disease

N.L. Khan, MD, MRCP; R. Katzenschlager, MD; H. Watt, MSc; K.P. Bhatia, MD, FRCP;
N.W. Wood, MD, PhD, FRCP; N. Quinn, MD, FRCP; and A.J. Lees, MD, FRCP

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Olfaction differentiates parkin disease from early-onset parkinsonism and Parkinson disease

N. L. Khan, R. Katzenschlager, H. Watt, K. P. Bhatia, N. W. Wood, N. Quinn and A. J. Lees

Neurology 2004;62;1224-1226

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VIDEO

Dopaminergic dysfunction in unrelated, asymptomatic carriers of a single *parkin* mutation

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Parkinson's Disease Is Not Associated with the Combined α-Synuclein/ Apolipoprotein E Susceptibility Genotype

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