Variation in Parkinson's disease: age, gender, genotype and phenotype correlations in early onset disease.

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Declaration of authorship: I, Naveed Malek, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the relevant places in my thesis.

Dedication: This work is humbly dedicated to all those men and women who have suffered at the hands of early onset Parkinson's disease (EOPD), who have to had live with this condition every day of their productive lives and beyond, recognizing the toll it has taken not only on their personal lives but also on the lives of their loved ones.

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Table of Contents:

Abstract:

Tracking Parkinson's -The PRoBaND study (Parkinson's Repository of Bio samples and Networked Datasets)

There is a wide variation in the phenotypic expression, progression rates, therapy response and complications in Parkinson's disease (PD). The primary research objective in this thesis was to analyse the variation in the 4 domains of phenotypic expression i.e. motor, non-motor, cognitive, and quality of life in a subset of early onset Parkinson's disease (EOPD) patients from the PRoBaND study, in the United Kingdom. The secondary objective was to explore the factors responsible for this variation or heterogeneity in the clinical characteristics.

Linking genotypes with phenotypes, besides evaluating environmental risk factors and iatrogenic influences, represents one mechanism of understanding this variation in the phenotypic expression of PD.

We found subtle but significant variation across all domains of symptoms examined in this thesis by classifying patients into groups according to motor subtype, gender, age at diagnosis and heritability of the parkinsonian trait, despite statistically insignificant differences in risk factors such as head trauma, exposure to pesticides (including herbicides, insecticides, fungicides and fumigants), heavy metals, caffeine and a past history of oophorectomy (in females) with the exception of smoking ($p=0.046$) and exposure to solvents, which were more common in males compared to females ($p<0.001$).

There were differences in the prevalence of motor symptoms such as balance problems being more prevalent in the postural instability gait difficulty (PIGD) subtype compared to the tremor dominant PD (TDPD) and 'Mixed' motor subtypes both subjectively ($p<0.001$) and objectively ($p<0.001$). Other axial problems such as speech difficulties and freezing were also more prevalent in those with the PIGD phenotype compared to the other motor subtypes both subjectively ($p=0.004$, $p<0.001$) and objectively ($p=0.002$, $p<0.001$). There was also variation in the prevalence of motor complications such as dyskinesia ($p<0.001$) and dystonia ($p=0.020$), being more prevalent in the PIGD subtype compared to other motor subtypes.

The prevalence of certain non-motor symptoms such as pain (p=0.022) and features of gastrointestinal dysfunction e.g. prandial bloating (p=0.024) and constipation (p=0.022) were more commonly reported by females compared to males.

There were also differences in the prevalence of cognitive impairment (p=0.049) and neurobehavioural characteristics such as anxiety (p=0.002) and depression (p=0.006), after the diagnosis of PD, being more prevalent in PIGD compared to other motor subtypes.

Finally, these differences contributed to the variation in the independence of activities of daily living scores which were lower in those with the PIGD phenotype compared to other motor subtypes ($p<0.001$).

There were some differences in exposure to environmental risk factors for PD but not sufficient to explain all the variation. Iatrogenic influences from drugs contributed in part to the phenotypic variation. 10% of the cases in the EOPD cohort tested positive for mutations in one of three genes screened i.e. *LRRK2, GBA* and *Parkin;* their DNA remains banked and there is scope to test these cases for mutations in other genes, relevant to PD, in the future. There were too small numbers of cases in each subgroup to draw definite conclusions about the exact influence of genes on the overall phenotypic variation but differences between *Parkin* mutation carriers and gene test negative 'controls' such as early age of onset and long disease duration were obvious.

PRoBaND is linked to other similar research studies in the UK, with the stated aim of sharing datasets, in the hope that larger numbers of patients and their DNA samples will increase the power, in statistical terms, to test hypotheses about the role of genetic markers in influencing the course and expression of symptoms.

Our current understanding of PD as a complex trait suggests both genetic and environmental influences (including iatrogenic factors if patients are treated) play a role in the phenotypic expression of this condition. A lot more remains to be explored to improve our understanding of the finer details and molecular mechanisms underlying the variation in this disease.

List of abbreviations:

ADL: Activities of daily living

CONSORT: Consolidated Standards of Reporting Trials

CRF: Case report form

CSF: Cerebrospinal fluid

DNA: Deoxyribonucleic acid

EDS: Excessive day time sleepiness

EOPD: Early onset Parkinson's disease

FOG: Freezing of gait

GBA: Glucocerebrosidase gene

GCSI: Gastroparesis cardinal symptom index

ICD Impulse control disorders

IRLSSG: International restless legs syndrome study group

JOPD: Juvenile onset Parkinson's disease

LEDD: Levodopa equivalent daily dose

Leeds SAA: Leeds self-assessment of anxiety scale

Leeds SAD: Leeds self-assessment of depression scale

LEU: Levodopa equivalent units

LOPD: Late onset Parkinson's disease

LRRK2: Leucine rich repeat kinase 2 gene

MDS-UPDRS: Movement disorder society unified Parkinson's disease rating scale

NACP: Non A-beta component (NAC) precursor

NMS: Non-motor symptoms (of Parkinson’s disease)

NMSS: Non-motor symptom scale

Parkin: Parkin gene

PDQ8: 8-item Parkinson's disease questionnaire

PDQSI: Parkinson's disease questionnaire summary index

PDSS: Parkinson’s disease sleep scale

PIGD: Postural instability gait difficulty

PINK1: PTEN induced putative kinase 1 gene

PRISMA: Preferred reporting items for systematic reviews and meta-analyses

PRoBaND: Parkinson's Repository of Bio samples and Networked Datasets

PSG: Polysomnography

QOL: Quality of life

QUIP: Questionnaire for impulsive-compulsive disorders in Parkinson's disease

RBD: Rapid eye movement (REM) sleep behaviour disorder

RLS: Restless legs symptoms

SCOPA-Aut: Scales for outcomes in Parkinson's disease-autonomic

SNCA: Alpha-synuclein gene

TDPD: Tremor dominant Parkinson’s disease

UPDRS1: MDS-UPDRS part 1 score

UPDRS2: MDS-UPDRS part 2 score

UPDRS3: MDS-UPDRS part 3 score

UPDRS4: MDS-UPDRS part 4 score

UPSIT: University of Pennsylvania smell identification test

List of tables

List of figures

Chapter 1. The natural history of treated Parkinson's disease

In 1817 James Parkinson described the first cases of a disease that now bears his name but he mainly described the motor features of a condition that can have manifestations across several domains including cognitive, autonomic, psychiatric and behavioural on a vast non-motor landscape. A broader description and understanding of how the disease unfolds over time and affects the body systems across the successive stages of this disorder is central to the process of treating patients afflicted by Parkinson's disease (PD) in order that all the medical issues that merit attention are addressed without losing focus on the core disabilities arising from the motor dysfunction.

The management of PD has changed over the last 200 years from Charcot's use of anti-cholinergics to treat his patients, as documented by his student Ordenstein in his doctoral thesis[1], to the breakthrough of finding the oral dopamine precursor 'gold drug' levodopa [2] following the discovery by Hornykiewicz of extensive reduction of dopamine in the brains of patients with PD [3].

While undoubtedly showing a beneficial effects on the core motor symptoms of bradykinesia, rigidity and tremor, some have argued that drug therapy has not changed the natural history of PD as mortality from the condition is not affected, there is no evidence that drug therapy can delay the onset of non-motor features such as dementia and even the response to some motor features such as falls is negligible[4]. Perhaps the finding that levodopa has limited beneficial effects on the non-motor symptoms of PD could be related to the involvement of biochemical pathways besides the dopaminergic neurotransmitter system in these manifestations of the condition[5]. The central pathology in PD though, is related to the loss of dopaminergic neurons in the substantia nigra and the accumulation of alpha-synuclien in neurons has a key role in the neurodegenerative process but how this process begins is still a matter of debate. Mutations in the gene coding for alpha-synuclein can cause PD but this is very rare.[6] Even mutations in other genes that can cause a phenotype of PD account for

only a minority of the total cases of PD; the majority of cases being currently labelled 'idiopathic' PD. This may change in the years to come as our understanding of the pathogenic mechanisms evolves.

Systematic reviews are one way of gathering all the data and evidence from studies previously conducted to obtain a broader view of how the natural history of PD evolves while allowing for standard treatment to be instituted.

Parkinson's disease on a timescale: A systematic review.

Abstract

Background

Parkinson's disease is the second most common neurodegenerative disorder affecting the elderly. In common with other neurodegenerative disorders it is a progressive condition. The progression of the motor symptoms of Parkinson's disease with time is well documented. Non-motor symptoms usually emerge earlier on a time scale while cognitive problems emerge later. Disability in general increases with time and this may reflect cumulative deficits across motor, non-motor and cognitive domains.

Objective

To review the literature and summarize the data from large case series of both untreated and treated Parkinson's disease to evaluate the progression rates in motor disability, the prevalence rates in non-motor symptoms, the changes in mortality data and the quality of life scores in these patients on a timescale.

Data Sources

Publications selected were based on original papers that had been abstracted in major online medical databases.

Study Selection

Publications cited are those meeting inclusion and criteria described below that shed light on the variation in the progression of motor and the prevalence of non-motor symptoms in untreated and treated Parkinson's disease, the standardised mortality ratios and quality of life of these patients that were published between Sep 1966 and Jan 2013.

Data Extraction

Data were primarily extracted from peer-reviewed literature appearing on Medline, Embase and Cochrane library.

Data Synthesis

Searches were limited to humans studies published in English. Hand searches were performed if references in the papers cited other sources that were missed on electronic searching.

Conclusion

The data available from this systematic review has helped us simulate the natural history of Parkinson's disease. This can serve as a tool for comparison of the prospective data that will become available from current and future observational studies of Parkinson's disease.

1.1 Introduction

Parkinson's disease first described in detail by James Parkinson in the early nineteenth century is a relentlessly progressive neurodegenerative disorder[7]. More recently, there is increased recognition that the underlying pathology in PD involves extranigral structures causing a host of problems affecting multiple body systems across motor, non-motor and cognitive domains. It is widely recognized that clinical progression of PD is multidimensional and is a function of time. Age at disease onset, duration of disease and pharmacological treatment with levodopa and dopamine agonists to a large extent determine the evolution of the global disability in PD. However genetic influences also contribute to the variation in progression rates of PD and several monogenic causes of autosomal dominant and recessive PD have been described.

1.2 Methods

1.2.1 Criteria for considering studies for this review

i. Types of studies: A search for all studies describing 20 or more subjects with PD (clinically diagnosed PD patients, with or without controls, including treated and untreated cases) was performed. Both cross-sectional and longitudinal studies were included. Convenience samples and cohorts restricted by certain demographic factors such as age or institution were also accepted.

ii. Types of participants: Diagnosis of PD required confirmation by an experienced physician, from history, examination and relevant investigations. Alternative diagnoses were excluded e.g. progressive supranuclear palsy, multiple system atrophy, and parkinsonism secondary to cerebrovascular disease or drugs. The progression of the disease should have been described over at least 1 year. No age group was excluded.

iii. Types of outcome measures: The main outcome measure was the description of progression of motor and/or non-motor symptoms of Parkinson's disease with time whether

treatment was commenced or not. Mortality rates in PD, causes of mortality, quality of life in PD and monogenetic causes of PD were also evaluated.

iv. Search methods for identification of studies: Ovid databases (Medline, Old Medline, Embase, Embase Classic), were searched combining medical subheadings progression, Parkinson* disease using the boolean operator AND; limiting the searches to humans and English language. Duplicate searches were then removed. Separate searches were conducted in the Cochrane library. Hand searches were performed for studies cited under references of review articles that were not located with electronic searches of databases mentioned above.

v. Scope of the review: Natural history of PD is affected by treatment with levodopa and progression rates in studies after 1967 when levodopa became available as the standard medical treatment will reflect treatment effects. Long term data on untreated cases post 1967 is very limited but where available is included. Studies in selected populations e.g. elderly are included. Surgical interventions are not considered as they are beyond the remit of this review. The focus of this review remains the change in symptoms as a function of disease duration hence studies not indicating disease duration are excluded.

1.2.2 Data collection and analysis

i. Selection of studies: We identified 3738 abstracts in Ovid databases Medline and Embase; 9 abstracts in Cochrane library combining MeSH disease progression, Parkinson* disease with the boolean operator AND. Limiting the abstract searches to English removed 209 abstracts; restricting the studies to humans rejected 485 abstracts and removing duplicate entries between the Medline and Embase databases removed 771 entries. Of the remaining abstracts, 136 abstracts fulfilled the inclusion criteria of case series containing 20 or more patients with Parkinson's disease and enough information available to document the progression of Parkinson's disease for at least 1 year.

ii. Description of studies: Case series containing 20 or more subjects in which sufficient clinical features were present to diagnose Parkinson's disease having excluded alternative diagnoses, reasonable descriptions of measures of disease progression were given, and enough information was available to assess response to treatment if this had been initiated, were summarized.

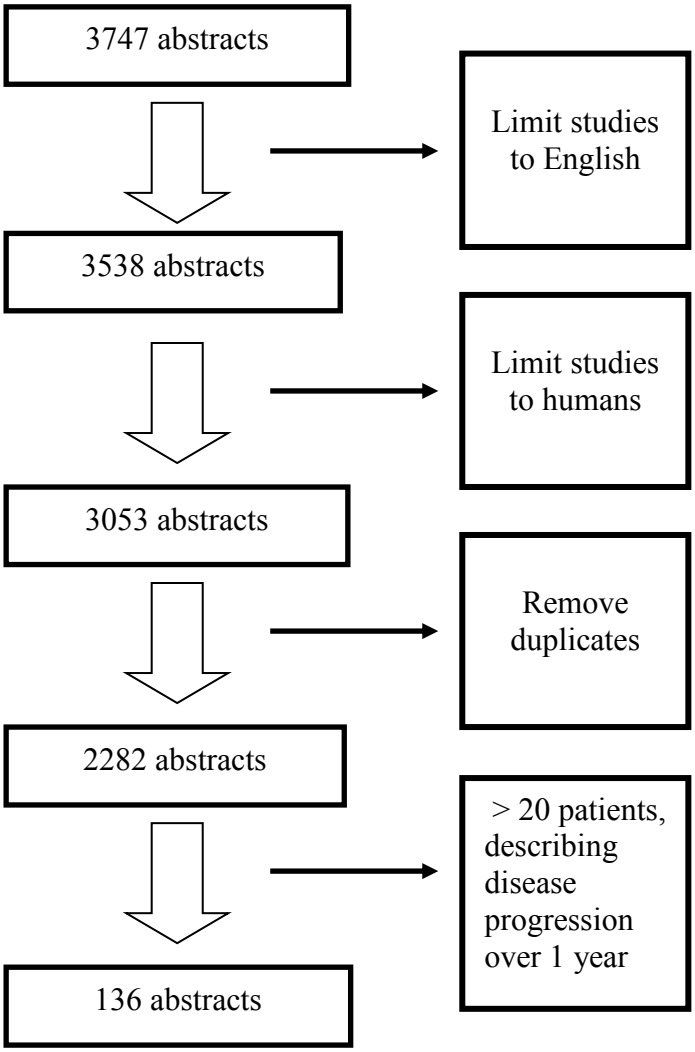

Figure 1.1 PRISMA diagram showing the process of paper selection for this systematic review.

1.3 Results

We summarize the key papers included in this review looking at 5 domains: cognitive, motor, non-motor, quality of life and morbidity/mortality in PD. Large scale studies of genetic cases of PD are limited but where available are included (all alphabetically listed in Tables).

1.3.1 The natural history of untreated Parkinson's disease

Since the introduction of levodopa by Cotzias in 1967 as a standard treatment for PD there are only a few studies which can describe the natural history of drug naïve PD patients for brief periods before standard treatment is commenced[2]. In the pre-levodopa era Hoehn and Yahr's seminal study of 856 parkinsonian patients seen at Columbia-Presbyterian medical center from 1949-1964 included 672 patients with 'primary parkinsonism' or idiopathic Parkinson's disease. The mean age at onset of disease was 55.3 years (range 17-89y) and men outnumbered women in a ratio of 2:1 (males 60.1%). Majority of the patients presented with a tremor (70.4%, 129/183) but non-motor presentations with pain, depression and paraesthesia were also recognized (13.1%)[8].

1.3.2 Variable progression in motor disability and prevalence of non-motor symptoms

1.3.2.1 Progression of motor dysfunction and prevalence of motor complications

a. Motor dysfunction

Measuring progression of any symptom, sign or disability over time and comparing the available data from other sources requires the use of an instrument (scale) that is valid, consistent and reproducible. More importantly the use of disease specific scales is preferred to generic scales due to different disease and population characteristics. We will present the data using scale(s) that are used in current clinical practice for each symptom as opposed to historical scales in order that a comparison of data from the various studies included in this review can be made. A general discussion for sources of variance in results follows at the end and where necessary after an analysis of the data in each domain. A brief description of what each scale purports to measure is given prior to presenting the results. Details on the use of the various scales mentioned is beyond the remit of this review but a reference will be provided for each scale so that the reader is directed to a more detailed description on its application.

Progression of motor symptoms has historically been measured using several scales including Hoehn and Yahr (HY), Columbia University rating scale and Webster to name a few. More recently Unified Parkinson's disease rating scale (UPDRS) and its adaptation Movement Disorder Society UPDRS (MDS-UPDRS) scales have become standard instruments for measuring motor symptom progression. Nevertheless HY scale for its simplicity continues to be used alongside the UPDRS and we have summarized our data from the studies included in this review using both instruments where available (Table 1.1). Those studies which lasted for less than 52 weeks such as TVP-1012 in Early Monotherapy for Parkinson's Disease Outpatients (TEMPO) (25 weeks) are not included as they do not meet the inclusion criterion for duration of observation but exceptions have been made where studies that have historical importance such as Earlier versus Later Levodopa Therapy in Parkinson Disease (ELLDOPA) (42 weeks) have been included.

The HY scale measures unilateral (HY-1) versus bilateral involvement (HY-2), normal balance versus postural instability with independence in daily activities (HY-3), and the ability to walk with assistance (HY-4) versus wheelchair dependency (HY-5). A modified version of this scale that is currently in use allows for intermediate stages 1.5 where axial involvement is present and 2.5 where disease is bilateral and mild but postural imbalance occurs.

HY staging records progression of motor disability on a categorical (non-parametric) scale as opposed to UPDRS Part 3 which records motor disability on a relatively continuous scale (parametric) from 0-108. UPDRS consists of 4 parts with the first 3 parts containing 44 questions each scored on a 0-4 scale (total for first 3 parts 0-176) and part 4 contains 11 questions and the total scores on part 4 can range from 0-23. Part 1 records mentation, behaviour and mood, part 2 records activities of daily living, part 3 records the motor disability and part 4 records complications of therapy. A modified version of this currently in use (MDS-UPDRS) extends the motor disability scale (part 3) to record the motor examination, in particular the tremor, in more detail so that the range of score extends from 0-136.

The annual rate of progression from the studies included in this review is 1.5 to 5.3 points on the UPDRS3 scale. (Table 1.1) The variation is accounted for partly by the fact that dopaminergic treatments have been commenced in these patients at various times after diagnosis singly or in combination. This pattern reflects standard clinical practice wherein

patients are commenced on treatment based on patient and physician preference, usually when motor disability becomes significant, and this point in time varies. Most of the evidence about annual rates of progression in the post levodopa era comes from drug trials which have a placebo arm and in particular those trials conducted after 1987 when the UPDRS scale, the most commonly used research tool to evaluate drug treatments in PD, in its current form came into use. In the pre-levodopa era one of the most remarkable pieces of evidence comes from Hoehn and Yahr's original case series (n=672) which in contrast documented the progression of motor disability step-by-step on the HY scale in years. The average duration in stage 1 was three years, stage 2 six years, stage 3 seven years, stage 4 nine years and stage 5 fourteen years[8]. In the post levodopa era a study of a similar magnitude in terms of longitudinal follow up is the Sydney multicenter study (n=126). From this longitudinal study the reported average times spent in HY stage 1 and stage 2 (by those who entered the study at these stages, 65.8%) was 3.5 y, in stage 3 was 7 years and in stage 4 was 6 years.(Table 1.1)

No statistically significant differences were found comparing the 15 year HY staging data from the Sydney mulitcenter study (patients treated with levodopa) with the 15 year data from the original Hoehn and Yahr's pre-levodopa era study ($p > 0.05$) suggesting that although levodopa improves symptoms in the first few years, the disease probably continues to progress at the same rate with or without levodopa [9].

As opposed to real time data computational Markov modelling to estimate the step-by-step progression rates from H&Y stages I to II, II to III, and III to IV or V has also been used in newly diagnosed cases (n=71).Stages IV and V were grouped together because of the sparseness of cases. Annual progression rates were first calculated and the inverse of each progression rate was then used to calculate the average time spent in stages I, II and III. The average duration in stages I was 2.8 years, II was 6.6 years and III was 1.4 years. The average delay time until progression to stage IV or V was therefore about 11 years[10] compared to 16 years in the Hoehn Yahr series[8].

The rate of progression of motor disability as well as age of onset is prognostically important and is one of the factors that have an impact on the quality of life in PD. There was an increased hazard ratio for death (1.4), disability (2.7) and cognitive impairment (4.3) with each 10 unit increase on the UPDRS scale. Age at study entry increased the hazard ratios to 49.1 for death, 4.76 for disability and 90.0 for cognitive impairment at age 60 years [11]. Earlier onset of postural instability (HY stage 3) reflecting a relatively faster rate of

progression had a significant impact on the quality of life in a representative study of PD patients (n=132) [12].

Data from studies using instruments that are not used in current clinical practice/research protocols such as University of California Los Angeles (UCLA) and New York University (NYU) scales have not been included for purposes of comparison.

Based on motor phenotypes those presenting without tremor also called postural instability gait difficulty (PIGD) have a faster rate of progression of motor disability [8, 13, 14].

Table 1.1 Progression of motor dysfunction in Parkinson's disease with time (papers listed alphabetically in table).

Study (Year)	**N**	**UPDRS 3*** (annual increment)	**Time spent in HY stages**				
			HY 1	HY2	HY3	HY4	HY5
Alves et al (2005) [15]	232	3.3		5.1-9.3y			
Hely et al (1999) †[16]	149		3.5y‡	7y‡	6y‡		
Hoehn et al (1967) [8]	183		3y†	6y†	7y†	9y†	14y†
Jankovic et al (2001)[13]	297	1.39§					
PSG (1996) ¶[17]	66P	5.3					
Liou et al (2008) [10]	37		2.8y	6.6y	1.4y		
Louis et al (1999) [18]	237	1.5 -3.6					
Lopez et al (2010) [19]	43L 21N	3.9\\ 2.9\\					
Marttila et al (1977) [20]	419		2.9y	2.6y	2y	4.2y	
PSG (1993) **[21]	57P	3.62					
PSG (2004) ††[22]	70P	5.2 ‡‡					
Schrag et al (2007) [23]	145	3.3					
Shults et al (2002)£ [24]	16P	5					

N=number of cases, UPDRS3= Unified Parkinson's disease rating scale Part 3, *= annual increase, HY= Hoehn Yahr stage , †= Sydney Multicenter study, ‡ median , PSG=Parkinson Study Group, ¶ =Lazebamide study, P = in placebo arm, L = L-DOPA arm, N= non-LDOPA arm, § for 'off' state , \\ after 1st year, ** DATATOP study, †† ELLDOPA study, ‡‡ 42 weeks , £ Coenzyme Q10 study

b. ***Motor complication rates***

Motor complications inevitably occur in the later stages of PD and in fact dyskinesias occurring with levodopa are considered to be one of the characteristics of idiopathic Parkinson's disease. Dyskinesias are involuntary abnormal movements which can affect any part of the body. This term encompasses both choreiform and dystonic movements. These may be related to dopamine levels in the body such as with 'peak dose dyskinesia' but in the later stages of PD this relationship becomes more complex. Freezing or motor block is an episodic inability (usually lasting seconds) to generate effective stepping in the absence of any known cause other than parkinsonism or high-level gait disorders[25]. Rapid changes from an 'on' state when medication is working well to 'off' state when medication effects have largely dissipated are called 'on' 'off' fluctuations while a more gradual transition from 'on to 'off' is called wearing 'off'. Motor complications can be recorded and their severity assessed in individual patients with UPDRS or MDS-UPDRS part 4. This part of the UPDRS records dyskinesia , 'on' 'off' fluctuations and wearing 'off' but does not record freezing which is recorded in part 3 of the scale. Data from cross-sectional studies is useful when determining the prevalence of these complications in a population (Table 1.2). Prospective studies have shown that motor complications increase with disease duration and are also related to initial treatment (levodopa vs. others) (odds ratio OR 3.87), age at onset (OR 0.90), and sex (OR 12.87) [26].

Table 1.2 Prevalence of motor complications of the disease on a time scale (papers listed alphabetically in table).

Study (Year)	N	Dur.	Dyskinesia	FOG	'On' 'Off'*	Wearing 'off'
Hauser et al (2007)[27]	42[R] 27[L]	10y	52.4% 77.8%			62.5%[†] 72.0%[†]
Hely et al (1994)‡ [28]	62[NL] 64[L]	5y	27.4% 54.7%			41% 37%
Holloway et al (2004) [29]	151[P] 150[L]	4y	37% 81%	56% 38%	10% 12%	71% 94%
Korczyn et al (1997) [30]	168[R] 167[B]	3y	7.7% 7.2 %			
Lopez et al (2010) [19]	43[L] 21[N]	3y	34.9% 4.8%	16.3% 0%	34.9% 0%	
		5y	51.2% 9.5%	30.2% 4.8%	65.1% 19%	
		7y	53.6% 35.7%	42.9% 42.9%	82.1% 37.5%	
		10y	84% 46.2%	76% 61.5%	96% 92.3%	
Montastruc et al (1994) [31]	29[L] 31[N]	3y	48.3% 12%			34.5% 40%
Poewe et al (1986) [32]	35[L]	6y	54%		6%	52%
Rascol et al (2000) [33]	179[R] 89[L]	5y	20% 45%	32% 25%		23% 34%
Rinne et al (1989) [34]	25[L] 5[l] 27[Ll] 20l[L]	4y	64% 0% 19% 20%	16% 0% 14% 20%		52% 0% 7% 20%
Ruiz et al (2004) [35]	36[L] 23[N]	3y	5% 25%	8%	15% 10%	
		5y	40% 20%	27%	45% 20%	
Ruiz et al (2012) [26]	25[L] 20[N]	7y	60% 45%	52% 35%	88% 50%	
		10y	72% 50%	76% 60%	96% 85%	

N= number, Dur= duration in years, FOG=freezing of gait, R= Ropinirole, L=Levodopa, ‡ Sydney multicenter study, NL= non-levodopa medication, P=Pramipexole, B=Bromocriptine, l=lisuride * 'on' 'off' includes 'off' period dystonia † mild wearing 'off'

1.3.2.2 Non-motor symptoms of Parkinson's disease on a timeline

Although James Parkinson may not have recognized the importance of non-motor symptoms (NMS) in the disease that bears his name, describing Paralysis Agitans "with the senses and intellect being uninjured", these symptoms are very common in PD. 98.6% of 1072 consecutive patients with PD in the PRIAMO (Parkinson and non motor symptoms) study reported the presence of non-motor symptoms[36]. These symptoms affect patients' quality of life, perception of disability levels [37] and most importantly can precede the diagnosis of PD by years which may provide an important time window for neuroprotective interventions (Table3).

Non-motor presentations of PD have been recognized for a long time [8] and are perhaps more common than previously thought. In 433 cases with pathologically proven PD, 91 (21%) had exclusively non-motor symptoms at presentation to their general practitioner. Of the NMS, pain was the most frequent, seen in 53% of these cases, urinary symptoms were present in 16.5%, depression or anxiety in 12.1% [38].

a. Pre-motor symptoms

A concept of pre-motor PD has emerged from imaging and pathological evidence of cell loss in the nigrostriatal dopaminergic system preceding the diagnosis of PD. By the time patients fulfil the diagnostic criteria for the diagnosis of PD, which are biased towards motor symptoms, there may be degeneration of about 50-60% of neurons in the substantia nigra[39]. The duration of the premotor phase following the onset of neuronal cell death is variable, but from pathological and radiological estimates has been considered to be between 3 and 5.5 years although in individual cases this can be more than a decade[40-43].

Table 1.3 Pre-motor symptoms in Parkinson's disease (papers grouped alphabetically in table).

Study (Year)	**Olf.** %/OR (yrs.*)	**RLS/EDS** OR (yrs.*)	**RBD** % (yrs.*)	**Constip.** OR (yrs.*)	**Auto.** OR (yrs.*)	**Dep/Psy** OR (yrs.*)
Abbott et al (2001) [44] N=6790				2.7-4.5 (12y)		
Gao et al (2011)[45] N=107,668				5m, 2.1f (6y)		
Savica et al (2009) [46] N= 202				3.0 (>20y)		
Haehner et al (2007) [47] N=30	7% (4y)					
Ponsen et al (2004) [48] N=361	10% (2y)					
Ponsen et al (2010 [49] N=361	12% (5y)					
Ross et al (2008) [50] N=2,267	OR 5.2 (4y)					
Olson et al (2000) [51] N=92			14.1% (3y)			
Postuma et al (2009) [52] N=93			16% 5y			
Schenck et al (1996) [53] N=29			38% (13y)			
Gao et al (2007) [54] N=32,616					3.8[§] (14y)	
Fang et al (2010) [55] N=279,958						2.7 (11y)
Santamaria et al (1986) [56] N=34						14.7 % (5y)
Schuurman et al (2002) [57] N=1358						3.1 (10y)
Shiba et al (2000) [58] N=392						1.4[d], 2.1[a] (5y)
Taylor et al (1999) [59] N=148						3.0 (22y)
Abbott et al (2005) [60] N=3078		3.3 (8y)				

Olf= olfactory dysfunction, RLS= Restless legs syndrome/ EDS= excessive daytime sleepiness, RBD= REM sleep behaviour disorder, Constip=constipation, Auto= Autonomic symptoms, Dep/Psy = Depression/Psychosis, yrs.*= years before diagnosis of PD, N=number of subjects, OR=odds ratio, § erectile dysfunction, m= male, f=female , d= depression, a = anxiety

Large prospective population-based registries have provided valuable information on the odds ratio of developing Parkinson's disease with non-motor symptoms such as olfactory loss that predate the motor symptoms and may be a useful screening tool to detect those at high risk for development of PD in later life.

Olfactory dysfunction was found to increase the odds ratio of developing PD in the next 4 years by 5.2 in the large Honolulu-Asia Aging (HAA) study (n=2,267) [50] and roughly about 1 in 8 with olfactory dysfunction in another series (n=361) went to develop PD over the next 5 years [49] . Nevertheless not all those who have idiopathic olfactory dysfunction went on to develop PD. Some have therefore argued diagnosing premotor Parkinson's disease using a two-step approach combining olfactory testing and dopamine transporter (DAT SPECT) imaging [61].

Another report emerging, from the HAA study (n=3078, age 71-93y), reported the risk of developing Parkinson's disease in men with excessive daytime sleepiness was considerably higher than in those without (odds ratio, 3.3) (95% CI = 1.4 to 7.0; p = 0.004) after 8 years [60].

Constipation has also been thought of as a pre-motor symptom in Parkinson's disease even though this is a common symptom in otherwise normal people and depends a lot on prevalent dietary patterns. In the Honolulu Heart Program, follow up of 6790 men aged 51-75 years, over a 12 year period, showed the incidence of developing Parkinson's disease increased with decreasing numbers of bowel movements per day. Men with <1 bowel movement/day had a 2.7-fold excess risk of PD versus men with 1/day (95% CI: 1.3, 5.5; p = 0.007) and this risk almost doubled when compared with men with 2/day (95% CI: 1.7, 9.6; p = 0.001) [44]. Other studies have also suggested a 2-5 fold increased risk of developing PD up to 20 years later in those with otherwise unexplained constipation compared to those with normal bowel habits[45, 46].

The association between preceding psychiatric disorders and PD using a case control design has also been evaluated. The frequency of psychiatric disorders was higher in cases than in control subjects at 5 years follow up; the odds ratio was 2.1 for anxiety disorders (95% confidence interval [CI] 1.3–3.2; p=0.001), 1.4 for depressive disorders (95% CI 0.8–2.6; p =0.3), and 2.4 for both anxiety disorders and depressive disorders occurring in the same

individual (95% CI 1.2–4.8; p= 0.02)[58].Other studies have also suggested an increased risk with odds ratio of 2-3 [55, 59].

REM sleep behaviour disorder preceding PD has been well documented [51]. The first comprehensive report of RBD as a predictor of neurodegenerative disease found that 38% of 29 male patients had developed a parkinsonian disorder 5 years after the diagnosis of idiopathic RBD [53]. A study of 93 patients with RBD diagnosed with polysomnography over 5 years showed that about 16% went on to develop parkinsonism[52].

Autonomic symptoms such as erectile dysfunction have been reported up to 14 years before developing PD. Men with otherwise unexplained erectile dysfunction were 3.8 times more likely to develop PD (n=200) during the follow-up than were those with very good erectile function(n=32,416) (relative risk = 3.8, 95% confidence interval: 2.4, 6.0; $p < 0.0001$)[54].

b. NMS after diagnosis of PD

As with motor dysfunction the proportion of patients experiencing non-motor symptoms is a function of time. (Table 1.4) A survey of a very large cohort of PD patients (n=10,101) showed that the percentage of people with PD experiencing NMS increased with the duration of the disease and there was an inverse correlation between the prevalence of NMS and quality of life (PDQ-8 scale) [62].

1. **Olfactory Dysfunction in PD**

Olfactory dysfunction is very common in PD but under reported by patients to their doctors. 72 % of PD patients were unaware of a smell disorder before testing; those who were aware had significantly lower test scores objectively compared to established norms[63]. Olfactory dysfunction is present in up to 90% of PD patients ultimately but comparison across studies is restricted with some studies using tests recording odour identification, odour discrimination, odour thresholds and variable cut-offs in the tests utilized.

There are several different tests to measure olfactory acuity in PD. It is important to understand what is being measured by a test under consideration as it is not possible to make

a direct comparison between two studies reporting results using different tests. Some olfactory tests are used for odour identification(OI) e.g. University of Pennsylvania Smell Identification test (UPSIT), some measure odour discrimination(OD) egg Düsseldorf Odour Discrimination Test, some record olfactory thresholds (OT) such as phenyl ethyl alcohol (PEA) or elecrogustatometry while others such as Sniffin Sticks® (Burghart GmbH, Wedel, Germany) detect all three. UPSIT (and its versions such as brief smell identification test, BSIT, and cross cultural smell identification test, CCSIT) and Sniffin' Sticks® test are the best validated tests in PD. For a detailed description of the test procedures and cut-offs please see Doty et al, 1984[64] and Hummel et al ,1997[65].

Previously olfactory dysfunction has been reported to be independent of disease duration [63], however, more recent reports suggest olfactory dysfunction correlates with disease duration [66, 67] . We included studies that provide a measure of disease duration as described in the inclusion criteria. (Table 1.4)

Table 1.4 Olfactory dysfunction in Parkinson's disease on a time scale (papers listed alphabetically in table).

Study (Year)	**N**	**Olfactory dysfunction (%)**				**Test**
		Duration (years) (mean or range as provided by authors)				
		0-5y	**5-10y**	**10-15y**	**15-20y**	
Boesveldt et al (2008) [68]	404	65%† (0-44y)				SS
Doty et al (1988) [63]	81			38 % [d] (12y)		UPSIT
Double et al (2003) [69]	49	82% (0-1y)				B-SIT
Haehner et al (2008) [47]	400		52 %[e] 45 % d (7y)			SS
Ramjit et al (2010) [66]	58			8 % [a] 8 % [b] 47 % [c] 33 % [d] (11y)		UPSIT
Ward et al (1983) [70]	72		39% (10y)			PEMEC Amyl acetate 4 odorants

N= number of cases , † 65% had deficits in olfactory identification while 42% had deficits in olfactory discrimination, SS-Sniffin Sticks, UPSIT-University of Pennsylvania Smell Identification test, B-SIT – brief smell identification test, * both anosmic and hyposmic, not age-adjusted for norms. a= mild hyposmia, b=moderate hyposmia, c=severe hyposmia, d=anosmia, e= any degree of hyposmia f= hyposmia or anosmia, PEMEC=phenylethylmethylethyl carbinol, 4 odorants: coffee, cinnamon, cherry and strawberry. Note: Studies not documenting duration of PD have been excluded.

The results of olfactory identification tests can be conditioned by cultural backgrounds, so it is important that the odorants used, as well as the odours suggested as possible answers, belong to the cultural background of the population which is tested. Therefore modifications of UPSIT for different ethnic populations have more recently been used in PD [67].

It is obvious that there are grades of olfactory loss from mild to severe hyposmia (also called microsmia) and anosmia. Most patients in PD have some degree of olfactory loss and there are reports that suggest that this is more likely to be in the mild-moderate end of the spectrum in early disease (<5 years) and severe hyposmia to anosmia later in the disease (> 10 years) [63] [66]. However, Double et al (2003) did not demonstrate any significant differences in olfactory dysfunction in the PD cohort across Hoehn Yahr stages I-III with an average disease duration of 1 year [69].

The cut-offs for defining hyposmia, normosmia and anosmia have varied from study to study both because of varying methodology used to detect odour deficits and the use of age matched controls to define limits of normal olfactory acuity that decreases with age.

2. Autonomic symptoms in PD

The range of autonomic symptoms that patients with PD experience is wide and includes gastrointestinal, cardiovascular and urinary dysfunction. These symptoms like other NMS are usually under reported. However when patients are asked to fill in questionnaires for autonomic symptoms the prevalence of these problems becomes evident. 89 % of PD patients (n=48) had at least one autonomic symptom from constipation, erectile dysfunction, bladder dysfunction, dysphagia and orthostatic dizziness, compared to 43% of healthy control subjects (n=32) in a questionnaire based survey ($p < 0.05$)[71]. Since then more structured instruments for recording the prevalence and severity of autonomic symptoms in PD have become available.

Scales for Outcomes in Parkinson's disease – Autonomic (SCOPA–Aut) is a validated questionnaire in PD for recording the presence of most autonomic symptoms. It contains 23 items in six domains: gastrointestinal functioning (7 items), urinary functioning (6 items), cardiovascular functioning (3 items), thermoregulatory functioning (4 items), pupillomotor functioning (1 item), and sexual functioning (2 items for men and 2 items for women). For further details on test performance and scoring please refer to Visser et al, 2004 [72].

Table 1.5 Autonomic symptoms in PD with disease duration (papers listed alphabetically in table).

Study (Year)	N	Dur.	Constip.	Bladder	Sexual	OS	Hyperh.	Sialo.
Edwards et al (1991) [73]	19* 79†	2.7y 8.4y	5% 35%					74% 69%
Korchounov et al (2005) [74]	532	6.2y	58.6%	22.4%	17.5%	29.5%	23.5%	
Magerkurth et al (2004) [75]	141	6.3y		45%	64% [m]	48%	46%	
Muller et al (2013) [76]	31* 140†	2.1y 2.4y	41.9% 33.6%	51.6% 36.4%		25.8% 30.0%		41.9% 37.1%
Ramjit et al (2010) [66]	58	11y	67.3%			10%‡		
Sakakibara et al (2001) [77]	115	6y	63% [f] 69% [m]	42% [f] 54% [m]	84% [f] 83% [m]			
Siddiqui et al (2002) [78]	44	8.3y	20.4%	68.2%	70% [m]	11-66%		52.3%
Singer et al (1992) [71]	48	8y	43.9 %	45.8%	60% [m]	21.9%		
Verbaan et al (2007) **[79]	420	10.5 y	50%	67% - 90%		4-56%		73%

N=number of cases, Dur. = duration of disease, ** those scoring >1 on the items for SCOPA-AUT, Bladder = bladder dysfunction (this includes urinary urgency) Sexual = Sexual dysfunction (this includes erectile dysfunction) OS = orthostatic symptoms (this includes syncope), Hyperh. = hyperhidrosis, Sialo. = sialorrhoea, m= males, f= females, *treated with dopaminergic medications, † drug naive, ‡ drop of systolic blood pressure of 20mm Hg on standing

Some of these autonomic problems may be either iatrogenic or exacerbated by anti-parkinsonian medication. Higher doses of dopaminergic medication were related to more autonomic problems in a study of 420 patients with PD [79]. Autonomic symptom severity was associated with other NMS including depressive symptoms, cognitive dysfunction, psychiatric complications, night-time sleep disturbances, and excessive daytime sleepiness (all p values < 0.01) [76].

The prevalence of autonomic symptoms in PD has been reported to be between 14-80% [80] but this is largely based on reports looking at one domain of autonomic dysfunction and

perhaps a true estimate is difficult to quantify into a single figure given that the frequency and prevalence of individual autonomic symptoms varies so widely (Table 1.5). The prevalence can vary from 25% for orthostatic symptoms to 51% for urinary dysfunction very early in the disease (2 years from diagnosis) to 56% for orthostatic symptoms and up to 87% for urinary dysfunction (nocturia) late in the disease (10 years from diagnosis) [76, 79] .

Constipation, which is a prominent pre-motor symptom as well, is variably defined in PD studies as being frequency of bowel movement less often than one per day (normal being greater than three stools per week) to less than three per week [73], but other studies also base their definition on stool consistency and frequency of straining. While constipation is not the only symptom of gastrointestinal dysfunction in PD, defecatory dysfunction (i.e. straining, incomplete evacuation) is reported as the predominant disorder of bowel function in PD rather than decreased frequency [73]. Other symptoms of gastrointestinal dysfunction including bloating, abdominal pain, nausea, vomiting, heartburn and dysphagia can also concomitantly present in 10-50% of patients [73].

Hoehn & Yahr (H&Y) stage, disease duration, age at onset and various therapy combinations all show significant correlations with autonomic dysfunction scores. However, PD patients show higher autonomic dysfunction scores than controls, even in the mild disease stages of the disease suggesting autonomic dysfunction as an inherent feature of PD [74].

3. **Depression/Anxiety**

Depression may precede or follow the motor symptoms of PD. Depending on the study designs, diagnostic criteria and the scale used for recording the symptoms, the prevalence of depression in PD is reported to be between 7 and 70% (Table 1.6), higher prevalence in hospital based cohorts may reflect a selection bias, towards the more severely affected individual requiring hospital placements. Minor depressive symptoms are present in a higher proportion of patients (70%) [81] in some series; the proportion of patients fulfilling DSM III/IV criteria for major depression is relatively lower in the range (13-30%) [82-86] in most series suggesting that the mild to moderate severity of the spectrum is the predominant phenotype encountered in clinical practice. In Starkstein's series major depression was commoner than minor depression however this was a hospital clinic based series as opposed to a community based series and it is argued that hospital based series in general can be

biased towards the more severe spectrum of the disease because those with minor symptoms would be less likely to seek medical attention in a hospital [85]. Most of the evidence about the prevalence comes from cross-sectional studies and it is difficult to make an exact comparison when different diagnostic criteria and instruments to detect depression and other psychiatric co-morbidities are employed. In Giladi's series (n=172) depression was associated with progression of the disease measured on the HY scale and earlier age at disease onset [87] and while the correlation between HY stage and depression was borne out in the larger (n=1449) German Study on the Epidemiology of Parkinson's Disease with Dementia (GEPAD) series but the correlation with disease onset didn't hold true. Females were more commonly affected than men with PD as in the general population. The importance in recognizing depression is that it is perhaps the variable with the most significant impact on the quality of life in PD [88].

Table 1.6 Psychiatric problems in PD with disease duration (papers listed alphabetically in table).

Study (Year) Number	**Dur.**	**Psychiatric co-morbidity in PD**					**Scale/ Criteria**
		Major depres.	Dysthymia	Minor depres.	Anxiety	Hypomania	
Bieliauskas et al (1989) N=33 [81]	9y			70%			MMPI- D, HDRS
Brown et al (1990) N=40 [89]	11y			25%	12.5 %		PSE-10, ICD-9
Cole et al (1996) N=31[82]	8y	13%	10%			6.5 %	DSM III R
Dissanayaka et al (2010) N=79 [90]	8.2y	11%	6%	6%	25% 3%		DSM-IV
Giladi et al (2000) N=172 [87]	11y	33%					DSM-IV
Leentjens et al (2012) N=132 N=118 [91]	6.4y 10y				9% 23%		HARS, DSM-IV
Mayeux et al (1981) N=55 [83]	9y	3.6%					BDI
Riedel et al (2010) N=1449 [92]	5.8y	25.2%					MADRS DSM IV
Schrag et al (2001) N=97 [84]	5.8y	19.6%*					BDI
Starkstein et al (2007) N=173 [85]	6.4y	30%	20%	10%			HDS, DSM IV
Tandberg et al (1996) N=245 [86]	8y	7.7 %					MADRS, BDI

Depres.= depression MMPI D Minnesota Multiphasic Personality Inventory D scale , HDRS- Hospital Depression Rating Scale, PSE 10 (Present State Examination, 10th edition), ICD 9- International system for classification of diseases , 9th edition, DSM III R= Diagnostic and Statistical Manual, 3rd edition, Revised ; BDI=Beck Depression inventory, * BDI>17 which signifies >borderline depression, DSM IV- Diagnostic and Statistical Manual, 4thedition, HARS- Hospital Anxiety Rating scale, HDS- Hamilton Depression rating scale , MADRS- Montgomery-Asberg depression rating scale. Note: Only studies utilizing set diagnostic criteria and rating scales included.

Anxiety is a common neuropsychiatric symptom in PD occurring in 9-25% of patients (Tables 1.6) and seems commoner in the later stages of disease (HY) however patients meeting criteria for Generalized Anxiety Disorder are considerably less. Anxiety also affects quality of life and adds to the complexity of PD [90].

4. **Restless Legs Syndrome (RLS)**

Restless legs is a common symptom in PD and can affect 0-25% of patients.(Table 1.7) Earlier studies may not be truly representative of the prevalence of RLS in PD due to possible misdiagnosis of nocturnal dyskinesia and akathisia that often occur in PD patients with RLS[93]. The use of validated criteria such as International RLS Study Group (IRLSSG) is therefore recommended. The Memory and Morbidity in Augsburg Elderly (MEMO) study provided a background rate of about 0.1%-10% in a community based cohort of people> 55 years without PD. This is significantly lower than the prevalence of RLS in PD. RLS symptoms are present in other parkinsonian disorders but much less frequent than in PD[94]. RLS is associated with periodic leg movements of sleep which contribute to the disrupted sleep pattern of these patients. The role of dopamine in the pathophysiologic mechanisms of RLS is supported by the improvement of symptoms with dopaminergic drugs.

Table 1.7 Prevalence of restless legs symptoms in PD with disease duration (papers listed alphabetically in table).

Study (Year)	**N**	**Duration of PD**				**Diagnostic criteria**
		0-5y	5-10y	10-15y	15-20y	
Bhalsing et al (2013) [94]	134	11.9% (4.6y)				IRLSSG
Gomez-Esteban et al (2007) [95]	114		21.9% (7.1y)			IRLSSG
Krishnan et al (2003) [93]	126		7.9% (5.4y)			IRLSSG
Nomura et al (2006) [96]	165		12% (10y)			IRLSSG
Ondo et al (2002) [97]	303	20.8% (>4.5y)				IRLSSG
Peralta et al (2009) [98]	113		24% (9y)			IRLSSG
Tan et al (2002) [99]	125		0% (5.5y)			IRLSSG

N=number of cases, International RLS Study Group (IRLSSG)

5. REM sleep behaviour disorder (RBD)

The diagnostic criteria for RBD, in the International Classification of Sleep Disorders (ICSD), include movements of limbs or body associated with dream mentation and at least one of the following: potentially harmful sleep behaviour, dreams that appear to be "acted out", or sleep behaviour that disrupts sleep continuity. PSG is not mandatory in these criteria. However, for two reasons studies with clinically probable RBD have not been included. First, reliable information from bed partners is not available in all patients. Second, patients with PD who are taking dopaminergic medication may show a variety of abnormal nocturnal motor and non-motor behaviours such as confusional states, hallucinatory episodes, and severe PLMS, which may be mistaken for RBD episodes in interviews. Previous studies

identified a poor sensitivity for RBD in clinical interviews in patients with PD as opposed to patients without PD [100, 101].

Table 1.8 Prevalence of REM sleep behaviour disorder (RBD) symptoms in PD with disease duration (papers listed alphabetically in table).

Study (Year)	**N**	**Duration of PD**				**Instrument used**
		0-5y	5-10y	10-15y	15-20y	
Gagnon et al (2002) [102]	33		58%* (7.7y)			PSG
Postuma et al (2008) [103]	36		58%* (5.7 y)			PSG
Romenets et al (2012) [104]	98		55%* (5.3y)			PSG
Sixel Doring et al (2011) [100]	457		46%* (8.7y)			PSG
Vendette et al (2007) [105]	34		53%* (5.2y)			PSG

*** based on polysomnographic (PSG) recordings. Note: Studies where diagnosis of RBD was not based on REM sleep phase documented on PSG associated with the movements are not included.**

Sleep dysfunction was even recognized by James Parkinson his essay the Shaking Palsy [7]. Night time sleep disturbances are common in PD, affecting up to 90% of PD patients[106] but only about half the patients had told their doctor of nocturnal problems[107].The most common sleep disorders reported by the patients with PD were frequent awakening (sleep fragmentation) and early awakening [108]. Nocturia, difficulty in turning over in bed, painful leg cramps, vivid dreams/nightmares, back pain, limb/facial dystonia and leg jerks are the main causes of nocturnal awakening in PD patients[109]. Both RLS and RBD disrupt sleep which can contribute to excessive day time sleepiness the following day.

The estimated prevalence of excessive daytime sleepiness (EDS) in PD patients varies from 20 to 50%. EDS may be due to the nocturnal disturbances, medication effects or a combination of both [110].

Finally to summarize the prevalence of NMS in PD is nearly universal. Non-motor symptom scale (NMSS) is a validated composite measure of the problems. The total NMSS scores were higher in patients with PD than those with other forms of parkinsonism that may look similar such as drug induced parkinsonism [111].

However many of the non-motor symptoms can be consequences of drug therapies as well and it may sometimes be difficult to disentangle the contribution of iatrogenic mechanisms from purely disease related phenomena in PD.

1.3.2.3 Prevalence of cognitive impairment in Parkinson's disease with time

In keeping with previous epidemiological studies recording the prevalence of other NMS in PD, the estimated prevalence rates of cognitive dysfunction in PD will vary depending upon the data collection methods, the population under study, scales used and the definition of dementia utilized in the study protocol (Table 1.9). The reported prevalence rates of dementia mostly from cross-sectional studies vary from 13 % at 3.5 years [112] to 78% at 17 years from disease onset [113] (DSM-III criteria). Two longitudinal studies have provided invaluable information about prevalence of dementia in PD from early to late stage disease and this data roughly reflects the figures available from cross-sectional studies. 17% of incident patients in a population-representative incident cohort developed dementia over 5 years. (CamPaIGN study, n = 126) [114]. The Sydney Multicentre Study found the prevalence of dementia increased with disease duration, from 24% at baseline to 80% at 20 years, but the evolution of dementia within PD occurred at around 70 years of age, regardless of the time of PD onset [115].

The association with increased disease duration was also confirmed in a study of young onset PD patients. However, dementia within PD could occur much earlier than 70 years (range 41-78 years). After a median disease duration of 18 years, cognitive impairment sufficient for a diagnosis of dementia was found in only 19% of patients (n=98) (13% of those younger than 60 years and 43% of those 60 years or older). Age, reflecting disease duration, was the most important factor for development of dementia, but female sex and positive family history of parkinsonism also had some positive predictive value [116].

The variation in the results of these 2 important studies reflects not only the differences in the tools used for the diagnosis of dementia (Tables 1.9a and 1.9b) but also the inherent differences of the populations studied.

Table1.9a Prevalence of dementia in PD with disease duration (papers listed alphabetically in table).

Study (Year) N	**Duration of disease**					**Instrument/ *Criteria***
	1y	2-5y	6-10y	11-19y	20y	
Aarsland et al (2001) [117] N=171		25.1% (4.2y)				MMSE, DRS, NPB *DSM-III-R criteria*
Aarsland et al (2003) [113] N=224			26% (9y)	78.2% (17y)		MMSE,UPRS m, GBSS *DSM-III-R criteria*
Balzer-Geldsetzer et al *(2011) N=604 [118]			13.8% (6.8y)			NPB *Emre criteria for PDD*
Evans et al (2011) N=132 [12]			35% (6.2y)			MMSE *DSM IV criteria*
Hobson et al (2004) [119] N=86		35.3% (4y)				CAMCOG *DSM-IV criteria*
Mahieux et al (1998) [120] N=81			23.4% (8.3y)			NPB *DSM-III-R criteria*
Marttila al (1976) [121] N=421			29% (7.2y)			*Celesia* & *Wanamaker criteria* (1972)
Mayeux et al (1992) [122] N=179			41.3% (5.7y)			NPB
Mayeux et al (1988) [123] *N=339*			11% (7y)			MMMSE *DSM-III criteria*
Melcon et al (1997) [124] N=51			15.7% (7.6y)			*DSM-III-R criteria*
Mindham et al (1982) [125] N=40			40% (9y)			NPB *GPRUIS criteria (1970).*
Mutch et al (1986) [126] N=252			11% (7y)			MMSE <5/10
Reid et al (2011) † [115] N=108	24% (baseline)	30% (3y)	38% (5y)	67% (10y)	80% (15y)	NPB *DSM IV criteria*

For legend see continuation of this table on next page (Table 1.9b)

Table 1.9b Prevalence of dementia in PD with disease duration (continuation of Table 1.9a)

Study (Year) N	**Duration of disease**					**Instrument/ *Criteria***
	1 y	2-5 y	6-10 y	11-19 y	20 y	
Schrag et al (1998)[116] N=149 of YOPD				19% (18y)		BTCT
Riedel et al (2008) [127] N=873			28.6% (6.7y)			MMSE, CDR, PANDA *DSM-IV criteria*
Snow et al (1989) [128] N=55			20% (8y)			MMSE<7/10
Sutcliffe et al (1995) [129] N=353			41.1% (8y)			MMSE <25/30
Wang et al (1996) [112] N=23		13% (3.5y)				CASI, CDR *DSM-III-R criteria*

N= number of patients at the start of the study .*DEMPARK study,† Sydney multicentre study, MMSE- Mini-Mental State Examination , DRS- Dementia Rating Scale, NPB- Neuropsychological battery, DSM-III-R Diagnostic and Statistical Manual 3rd edition Revised, UPDRSm- the mentation item from the mental subscale of the Unified Parkinson Disease Rating scale , GBSS- Gottfries-Bråne-Steen scale, PDD- Parkinson's disease dementia, CAMCOG- Cambridge Cognition Examination, DSM IV- Diagnostic and Statistical Manual 4th edition, MMMSE- Modified Mini-Mental State Examination , DSM-III Diagnostic and Statistical Manual 3rd edition, GPRUIS- General Practice Research Unit Interview Schedule criteria, BTCT- brief telephone cognitive test ¸ CDR – Clinical Dementia Rating Scale , PANDA- Parkinson Neuropsychometric Dementia Assessment (PANDA), CASI-Cognitive Abilities Screening Instrument.

1.3.3 Quality of Life in PD (QoL)

Measuring the QoL in Parkinson's disease in the past has been done with generic instruments such as The Barthel index and the modified Baecke questionnaire but these are not specific to PD and are a hindrance when comparing results from studies using different questionnaires. We have therefore included studies that have used a disease-specific measure.

The 39 item Parkinson's disease Questionnaire PDQ-39 and 8 item PDQ-8 have been specifically designed for use with individuals with Parkinson's disease and has shown validity and reliability. The PDQ-8 is derived from the 39-item. A higher score indicates poorer quality of life (for details on scoring please see Jenkinson et al, 1997 [130]). These scales also incorporate measures of activities of daily living (ADL).

In a prospective multicentre UK based study looking at self-reported quality of life in a cohort of 198 drug naïve PD patients over 18 months, the self-reported health status scores in all eight domains of the PDQ-39 and the overall PDQ-39 summary index worsened significantly ($p<0.01$) in patients left untreated [131]. Therefore patient preference about improving and maintaining quality of life may counterbalance the traditional physicians' perspective of delaying levodopa based treatments in the hope of delaying onset of motor complications like dyskinesia and 'on' 'off' fluctuations.

Disability, postural instability, and cognitive impairment had the greatest influence on QoL in Parkinson's disease [132]. In other studies QoL also depended on daily dosage of levodopa, disease duration, disease progression, sex, tremor scores, clinical fluctuations as measured by UPDRS part IV and the H–Y stage of disease. These factors taken together account for the variation in QoL scores [133,134].

Therefore, addressing all these factors that affect the patients QoL should become an important focus of treatment of PD [133].

Table 1.10 Variation in quality of life scores in PD with disease duration (papers listed alphabetically in table).

Study (Year)	N	Duration	HY / UPDRS 3	PDQ 8*/ PDQ39†
Carod-Artal et al (2007) [134]	144	6.6y	2	40.7†
Chapuis et al (2004) [135]	143	9.1y	2	36.5†
Cubo et al (2002) [136]	158	8.1y	2.2	48.8†
Gomez- Esteban et al (2007) [137]	110	7.6y	24.73 UPDRS 3	41.3†
Grosset et al (2007) [131]	198	4y	1.6	18†at baseline‡ 31†at 9 months‡ 43†at 18 months‡
Klepac et al (2007) [138]	111	5y	25 UPDRS 3	47†
Michalowska et al (2005) [139]	60	8y	2.7	32.1†
Navarro-Peternella et al. (2012) [140]	40	8.4y	2.5	35.45†
Rahman et al (2008)	130	12.1y	2.6	25.2 HY<2.5† 39.6 HY>3†
Schrag et al (2000) [132]	92	5.3y	2.35	30†
Slawek et al (2005) [133]	100	6.7y	2.6	34†
Zach et al (2004) [141]	141	11.9y	2.5§	35.3†
Zhao et al (2008) [142]	183	4.6y	2.3	27.5*

N=number of cases, Y= years, ‡ in drug naïve patients who remained without any treatment over 18 months (n=61), § between HY stage II and III.

1.3.4 Morbidity and Mortality

1.3.4.1 Standardised mortality ratios

The effect of Parkinson's disease on mortality, and whether anti-parkinsonian medication impacts this, has been long debated.

Standardised mortality ratios (SMRs) are estimated from the ratio of observed to expected number of deaths. Mortality rates in general in PD cohorts are greater than the general population when matched for age and gender (Table 1.11a).

In the pre-levodopa era (1949-64) Hoehn and Yahr's cohort of PD patients (n=672) the mean age of disease onset was 55.3 years, mean duration of illness was 9.7 years and SMR was 2.9 [8].

In the post-levodopa era Barbeau in one of the early studies found no significant difference in the SMR compared to the pre-levodopa era. However, most studies have found a reduction of death rate during long-term levodopa treatment and this effect can be seen even at 20 years post diagnosis [143].

Using regression modelling, to assess the impact of the timing of levodopa administration during the course of illness on mortality, while statistically controlling for other factors (i.e., patient selection for levodopa treatment, and independent predictors of survival), risk of death following initiation of levodopa was significantly reduced ($p < 0.001$), regardless of pre-levodopa duration of illness. At no point in time was levodopa treatment associated with increased mortality, arguing against levodopa toxicity [144].

Using a modified Gompertz function and data on mortality from 6 studies [116, 145-149] of SMR in PD and life expectancy in the UK from the Office of National Statistics, the calculated estimated age specific life expectancies in patients with PD compared with the general population were 38 (SD 5) years for onset between 25 and 39 years compared with 49 (SD 5) years; 21 (SD 5) years for onset between 40 and 64 years compared with 31 (SD 7) years; and 5 (SD 4) years for onset age > or = 65 years compared with 9 (SD 5) years [150].

Table 1.11a Mortality ratios (observed: expected deaths) in PD with disease duration (papers listed alphabetically in table).

Study (Year)	**N**	**Mortality ratio. Observed: Expected deaths (Duration of disease in years)**			
		< 5y	5-9 y	10-19 y	>=20 y
Barbeau et al (1976) [151]	80		2.4 (6y)		
Ben-Shlomo et al (1995) [148]	220				2.6 (20y)
Cedarbaum et al (1987) [152]	100			1.9 (16y)	
Curtis et al (1984) [153]	176		1.5 (6y)	2.59 (12y)	
Diamond et al (1989) [154]	54		1.8-2.2 (6y)		
Duarte et al (2013) [143]	273				1.39 (20y)
Ebmeier et al (1990) [155]	267	2.3 (3.5y)			
Hely et al (1999)* [16]	108			1.58 (10y)	
Hoehn & Yahr (1967) [8]	672		2.9 (9.4y)		
Herlofson et al (2004) [146]	245		1.5 (8y)		
Louis et al (1997) [156]	288		2.7 (8.8y)		
Marttila et al (1977) [157]	349		1.8 (7y)		
Morgante et al (2000) [147]	59		2.3 (8y)		
Rinne et al (1980) [158]	349	1.1-1.7 (2-3y)	1.9 (9y)		
Schrag et al (1998) [116]	JP=10 YOPD=139			2.0 (18y)	3.0 (24y)
Shaw et al (1980) [159]	178		1.5 (6 y)		
Sweet et al (1975) [160]	100	1.9 (5y)			
Tison et al (1996)† [149]	24		3.43 (5y)		

JP= Juvenile parkinsonism (age at onset <21y) YOPD= Young onset Parkinson's disease (age at onset<40y),*Sydney Multicentre study, † PAQUID study

Table 1.11b Causes of mortality in Parkinson's disease (papers listed alphabetically in table).

Study (Year) N	**Causes of death by system/disease (%)**						
	Respiratory disease*	Heart disease	Cancer	CVD	PD	Other	Unknown
Ben Shlomo et al (1995) N=220 [148]	16.4	23.1	4.6	16.9	-	39	-
Ben Shlomo et al (1998) N=624 [161]	4	26	12.5	10	40	7.5	-
Beyer et al (2001) [162] N=84	24	18	17	9.5	-	32.2	-
D'Amelio et al (2006) [163] N=59	27	35	7	20			
Fall et al (2003) [164] N=170	23	13	8	6			
Hely et al (1999) [16] N=108	30	21	19	16	5	6	3
Morgante et al (2000) [147] N=59	15	17	<1	13.5	-	10	-
Pennington et al (2010) [165] N=143	11	12	12	9	29	27	-

N=Number of cases, CVD= cerebrovascular disease, PD- Parkinson's disease , *Respiratory disease includes pneumonia.

1.3.4.2 Causes of mortality in Parkinson's disease

The 5 most common causes of mortality in PD are cardiovascular, respiratory, cerebrovascular disease, cancer and advanced stage PD combining the results from 8 studies reporting causes of mortality in PD. (Table 1.11b, Figure 1.2). Not surprisingly cardiovascular disease, reflecting the trend in the general populations from the Western world, was the most common cause of death in PD. This reflects the findings from a recent longitudinal 20 year study of PD as well [143] but results from other studies are variable (Table 1.11b).

The variation in the results partly reflects the different methods used when conducting mortality studies. Death certificates documenting proximate cause(s) of death are often prepared in a hospital or hospice setting not laced with research methodology, thus leading to a possible under reporting of the terminal diagnosis as underlying co-morbidities may be multiple, and in some cases a positive diagnosis given to the patient may not be proven at autopsy. The level of accuracy of the diagnoses documented on death are subject to variation in different countries and in different time periods within the same country reflecting different reporting patterns[162]. In a more general sense the underlying heterogeneity of the population and environments in which those populations live reflecting different life styles may also contribute to variation in e.g. the proportion of patients dying with cardiovascular disease.

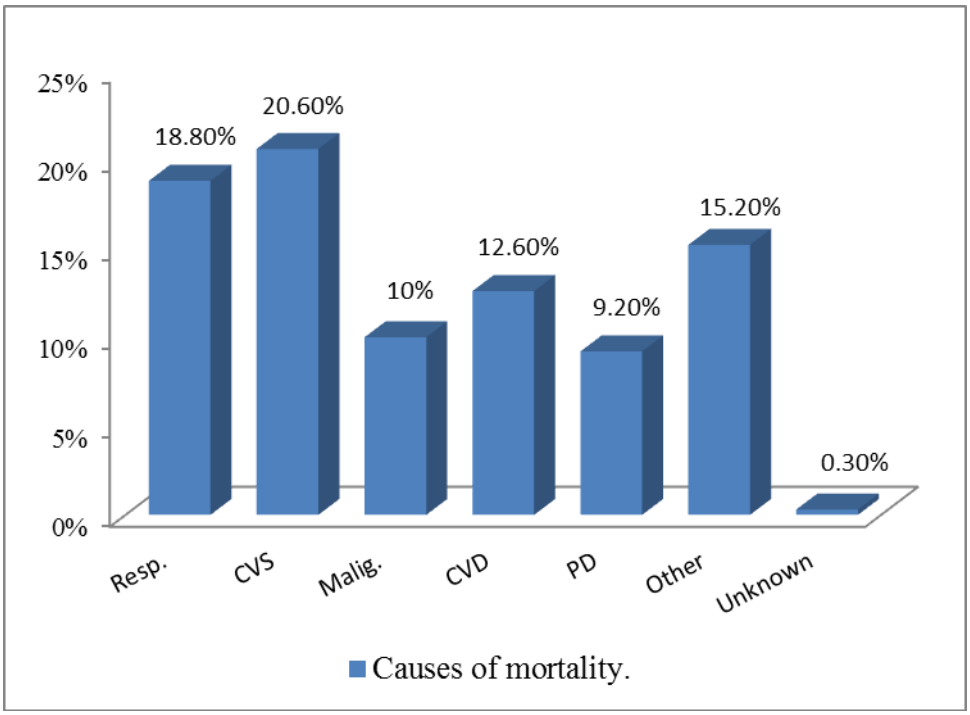

Figure 1.2 summarizes the data on mortality in Parkinson's disease from the 8 studies included in Table 1.11b.

1.3.5 Comparison with the Mendelian inherited forms of Parkinson's disease

The three most common mutations linked to PD are the autosomal dominant *GBA, LRRK 2* gene mutations and autosomal recessive *Parkin* gene mutations. *PINK1, DJ1* and *ATP13A2* gene mutations are rarer.

Homozygous mutations in the glucocerebrosidase gene (*GBA*) cause Gaucher's disease and heterozygous mutations are a risk factor for PD. A study evaluating the frequency of *GBA* mutations in British patients affected by PD (n=790) compared with normal controls (n=257), matched for age and ethnicity reported that the frequency of *GBA* mutations in PD patients (4.18%) was significantly higher ($p = 0.01$; odds ratio = 3.7; 95% confidence interval = 1.12-12.14) when compared to controls (1.17%). The phenotype of mutation carriers with PD comprised an early onset of the disease, the presence of hallucinations in 45% and symptoms of cognitive decline or dementia in 48% of patients. Pathological examination revealed widespread and abundant alpha-synuclein pathology in all *GBA* mutation carriers (n=17) who had autopsies. This large study with genotype/phenotype/pathological analyses showed that mutations in *GBA* are found in British subjects at a higher frequency than any other known Parkinson's disease gene [166].

Mutations in the *LRRK2* gene, coding for leucine rich repeat kinase 2, cause autosomal dominant Parkinson's disease. The *LRRK2* phenotype was defined on the basis of 59 motor and non-motor symptoms in 356 patients with *LRRK2*-associated PD and compared with the symptoms of 543 patients with pathologically proven idiopathic PD. A worldwide population study showed the frequency of the commonest *LRRK2* mutation, Gly2019Ser, was found in 1% of patients with sporadic PD and 4% of patients with hereditary PD; the frequency was highest in the Middle East and higher in southern Europe than in northern Europe. The risk of PD for a person who inherits the *LRRK2* Gly2019Ser mutation was 28% at age 59 years, 51% at 69 years, and 74% at 79 years. The *LRRK2* phenotype (n=356) showed motor symptoms (e.g., disease severity, rate of progression, occurrence of falls, and dyskinesia) and non-motor symptoms (e.g., cognition and olfaction) were more benign than those of idiopathic PD (n=543) [167].

A wide variety of mutations in the *Parkin* gene (PARK2) are responsible for autosomal recessive parkinsonism. The phenotype of *Parkin* gene mutation carriers is characterized by early onset (before age 40) of disease, marked response to levodopa treatment and levodopa-

induced dyskinesia. In 35 mostly European families with early onset autosomal recessive parkinsonism with *Parkin* gene mutations mean age at onset was 38 +/- 12 years however in many patients except for early age of onset, the phenotype is indistinguishable from that of idiopathic PD[168].

Table 1.12 Phenotypes of *LRRK2, GBA* and *Parkin* mutation carriers with Parkinson's disease (papers listed alphabetically).

Study (Year) N	**Gene**	**Age of onset** (mean)	**Rate of progression**	**Dystonia**	**Dopamine response**	**LID**	**Cognitive problems**
Healy et al (2008) [167] N= 321	*LRRK2*	58.1y	HY I: 4 y HY II : 7.2 y HY III: 9.4 y HY IV:12.6 y HY V: 15.6 y	42%	89% good 9% modest 3% poor	11% (5 y) 58% (8y)	23% (14 y)
Lucking et al (2000) [169] N=54	*Parkin*	32y	HY I: 11 y HY II : 19 y HY III: 26 y HY IV: 40 y HY V: 15.6 y	58%	72% good	77% (5y)	<1 % (17 y)
Neumann et al (2009) [166] N=31	*GBA*	52.7y	-	-	90% good	-	48% (12y)

N=Number of cases, LID= levodopa induced dyskinesia, *LRRK2*= Leucine rich repeat kinase 2 gene, *Parkin*= Parkin gene, *GBA*= Glucocerebrosidase gene, HY= Hoehn Yahr stage. Note: Studies including less than 20 cases not included as per study protocol.

1.4 Discussion

Our knowledge about the natural history of Parkinson's disease, until recently, came from studies which have used the date of presentation to the clinician as a starting point. These studies have therefore predominantly focussed on the clinical phase of the disease, when nearly 80% of dopamine stores have already been depleted [170, 171]. We know that Parkinson's disease has a preclinical and a premotor phase. To understand more about these phases of the illness we may have to rely on epidemiological studies using population based cohorts such as from the HAA study.

Whilst there are numerous reports documenting the progression of the motor symptoms of PD, there are not many longitudinal studies about the evolution of non-motor symptoms in this disease after the onset of motor dysfunction. However we know from previous studies that non-motor features such as hyposmia, constipation, erectile dysfunction, depression, daytime somnolence and REM sleep behaviour disorder, can precede the motor symptoms of PD, sometimes by many years (Table 1.3). A critical appraisal of these premotor symptoms suggest they could be used as biomarkers of a preclinical PD phenotype. Some studies have combined clinical, genetic and neuroimaging investigations to study the premotor phase in subjects at high risk for developing PD such as hyposmic individuals (Parkinson's Associated Risk Study) [172].

Cognitive dysfunction, although not recognized by James Parkinson, thought to be one of the most important features of PD in late stages is a function of time, chronologically analogous to the progression of motor disability. The most compelling piece of evidence for this comes from the longitudinal Sydney Multicentre study; over 20 years the proportion of patients developing dementia increased from 24% to 80% in surviving patients[115].

The rates of progression of motor, non-motor and cognitive symptoms vary in individuals but in general there is a cumulative disability and this invariably affects patients' quality of life. (Table 1.10)

The importance of predicting who develops PD cannot be more emphasized than by stating that if future neuroprotective drugs are going to make a difference in the relentless cascade of neurodegeneration that follows once the pathologic burden of alpha-synuclein aggregates in

the brain reaches a critical mass, these drugs will have to target a population of patients where the disease process is not yet entrenched.

Once PD is diagnosed it is well recognized by clinicians and patients that it is a progressive condition. In order to make management decisions regarding treatments offered to patients, clinicians assess and record the progression of motor disability so that medications are tailored to disease stage. Besides using validated scales, such as UPDRS, for recording disease progression, there have been efforts to find alternative methods to do this in vivo. Nuclear medicine imaging provides one such tool for measuring disease progression, assessing the rate of loss of presynaptic dopaminergic neurons although this relationship may not be linear due to the possible confounding effects of concomitant dopaminergic medications and therefore better biomarkers of disease progression are required.

The factors affecting the rates of progression of motor and non-motor symptoms have not been elucidated sufficiently to explain all the variation. Dopaminergic and other medications alter the natural history of PD as they decrease morbidity, mortality and improve quality of life [173] but the variation in progression is too large to be explained by medication effects alone. Environmental influences, besides drugs, and cultural differences may affect the subjective perception of disability but objective differences probably reflect the underlying heterogeneity of the populations studied. Although a perfect understanding of all the factors that underlie the variability in PD is limited, over the last 15 years our understanding of the genetic influences in PD has become clearer. While genetic causes account for only a small proportion of the total cases of PD, subtle genetic differences between individuals (single nucleotide polymorphisms) at several loci in the human genome probably explain a lot of the variation in the expression of PD. Gene-gene and gene-environment interactions may explain more of this variation than the influence of single genes e.g. polymorphisms in the *N-acetyl transferase 2* gene which codes for an enzyme responsible for the biotransformation of exogenous neurotoxins, results in a slower rate of metabolism of these detrimental influences from the body. This can lead to greater vulnerability to neurotoxins and to a higher susceptibility of PD [174].

Recent studies suggest genetic factors may also underlie the variability in cognitive and motor dysfunction [175]. Further genetic differences in the activities of dopamine metabolizing enzymes could influence both the individual response to therapy and the risk of early motor disturbances with MAOB and COMT inhibitors [176]. An argument can

therefore be made that patient heterogeneity reflecting genetic differences as well as environmental influences interacting with human genes may underlie the variability in other aspects of PD as well.

1.5 Conclusion

Although differences in methodologies and reporting of the results make a direct comparison of all studies not possible, it is quite clear from the evidence provided that progression of motor symptoms, signs and disability on a time scale is nearly universal in PD despite the individual variation. Non-motor symptoms are abundant and some precede the development of the motor-symptoms by years while others like cognitive problems show a cumulative prevalence, which on a chronological timeline would parallel the progressive motor disability that these patients experience, such that by 20 years the vast majority of surviving patients show scores on neuropsychological batteries compatible with a diagnosis of dementia.

Search strategy

I. For Medline and Embase

1. parkinson*.mp. [mp=ti, ab, ot, nm, hw, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]

2. disease progression.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, an, sh, tn, dm, mf, dv, kw]

3. 1 and 2

4. limit 3 to English language

6. limit 4 to human

8. remove duplicates from 5

II. For Cochrane Library using advanced search

MeSH parkinson* disease AND disease progression

Chapter 2. Alpha-synuclein as a molecular biomarker of Parkinson's disease

The official National Institute of Health (United States) definition of a biomarker is: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [177]. Clinical, genetic, blood and cerebrospinal fluid (proteomics, transcriptomics, metabolomics), and neuroimaging biomarkers may provide useful tools in the diagnosis of Parkinson's disease (PD) and in measuring disease progression and response to therapies[178]. The diagnostic value of these biomarkers lies in their ability to help differentiate PD from its mimics and to predict who is at a risk for PD. The prognostic role of clinical biomarkers such as the motor subtype and cognitive impairment in PD is recognized [114, 179] and the value of biochemical markers such as serum levels of advanced oxidized protein products that could participate in the development of parkinsonian neurodegeneration as a prognostic marker of PD duration is an area of potential research[180].

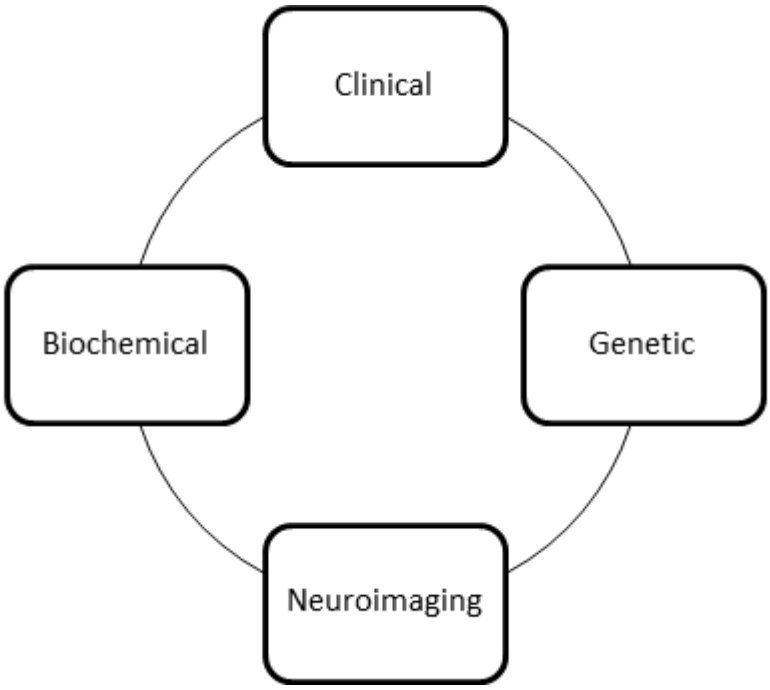

Figure 2.1 shows the categories of biomarkers for Parkinson's disease under investigation.

Biomarkers used as diagnostic tests, prognostic tools, or surrogate endpoints must not only have biologic relevance but also a strong linkage to the clinical outcome of interest [180].

i. Clinical biomarkers

a. Olfactory dysfunction

Olfactory dysfunction is near-universal in PD [181] and may predate the clinical diagnosis of PD by years. Recent data indicate that over 95% of patients with PD present with significant olfactory loss. Thus, olfactory dysfunction could be considered a reliable biomarker of the disease. There is poor performance of PD patients on odour discrimination, odour detection and odour identification tasks. As significant olfactory impairment is found early in the disease process, olfactory tests may be useful. However, it is not a specific bio-marker as olfactory dysfunction has been documented in other neurological disorders such as Alzheimer's disease, multiple sclerosis, Huntington's disease and old age [182].

b. REM sleep behaviour disorder

Rapid eye movement (REM) sleep behaviour disorder (RBD) is a parasomnia characterized by vivid dreams associated with simple or complex motor behaviours during REM sleep. The polysomnographic (PSG) features of RBD include increased electromyographic tone and dream enactment behaviour during REM sleep [183].

REM sleep without atonia (RWA) shows abnormal muscle activation during REM sleep during PSG without manifest behaviours may represent preclinical forms of RBD is more common in PD than RBD[102]. RBD or RWA is present in 25 to 50% of PD patients[184].

Probable RBD (based on informant response to the questionnaire) is more frequent in PD than essential tremor and could suggest the diagnosis of PD in a tremulous patient[185] but RBD is not specific to PD and can occur concomitantly with other alpha-synucleinopathies including Lewy body dementia and multiple system atrophy[186].

c. Restless legs syndrome

Restless legs syndrome (RLS) is marked by a tendency to keep moving the legs accompanied by a premonitory urge and dysesthesias that occur at rest and are alleviated by movement. Symptoms occur in a circadian manner being most prominent in the evening hours and when lying in bed to sleep[184]. Despite the observations suggesting that RLS may be a dopaminergic deficiency state given that it responds to dopamine agonists, it is clear that RLS differs pathologically from PD as the dopaminergic dysfunction does not involve neuronal degeneration and likely involves dopaminergic pathways other than the nigrostriatal pathway that is involved in PD[187]. RLS is also not specific to PD and can be associated with several other disorders such as such as iron deficiency, pregnancy, medications, chronic renal failure, leprosy, peripheral neuropathy and systemic sclerosis[188-192]. Although the symptoms of RLS are relatively common in patients with PD; however, at present there is no evidence that RLS symptoms early in life predispose to the subsequent development of PD. There is some evidence to suggest that RLS may be a secondary phenomenon, in some cases this may bear a relation to their low ferritin levels. [97].

d. Constipation

Gastrointestinal symptoms that are significantly more prevalent in PD patients include dry mouth, drooling, dysphagia, constipation and defecatory dysfunction. Constipation and defecatory dysfunction precede motor manifestations and can be therefore be included amongst other non-motor symptoms such as hyposmia and RBD that can be considered as bio-markers of pre-motor PD. Gastroparesis symptoms may also precede motor manifestations but like other autonomic manifestation these symptoms are also not specific to PD [193].

e. Family history of PD

Family history is consistently identified as an independent risk factor for PD in systematic reviews and meta-analyses [194, 195] . There is a broad range of risk estimates reported

ranging from 4 to 40 times higher amongst relatives of people with PD [59, 196-198]. It is likely that biases in collecting data and variation in study designs have contributed to this large range of reported risk, however, these studies all concur with the multifactorial hypothesis of PD, in which inherited genes interact with environmental influences to determine the overall risk of an individual developing PD over his lifetime.

ii. Genetic biomarkers

Genetic biomarkers in the form of disease causing genes or risk-modifying variants, can define the risk of an individual developing a disease, and allow stratification of patient populations according to the underlying molecular defect [199]. However, the case load of monogenic causes of PD is small overall and the aetiology in the majority of patients with sporadic or hereditary forms of PD remains unknown in most populations [200]. The relationship of genotype to phenotype in complex traits such as Parkinson's disease is also not straightforward as the heterogeneity in the clinical expression of PD is too large to be explained by a mutation in a single gene and perhaps there are other yet unidentified biological influences besides environmental factors that modify phenotypic expression. Nevertheless in individual cases and families with PD the genetic carrier status of the proband can be tested to inform about prognosis and inheritance risks.

iii. Neuroimaging biomarkers

Midbrain/nigral structural abnormalities can be demonstrated *in vivo* with both trans cranial sonography (TCS) and diffusion tensor magnetic resonance imaging (DT-MRI) while positron emission tomography (PET) and single photon emission computed tomography (SPECT) ligands exist to demonstrate dopamine terminal dysfunction[201].

To confirm the diagnosis of degenerative parkinsonism, the PET ligand [18F]-DOPA for estimating dopaminergic neurotransmission by estimating striatal aromatic amino acid decarboxylase (AADC) activity , SPECT ligands ^{123}I fluopane and βCIT for dopamine transporter imaging and 11C- or 18F-dihydrotetrabenazine PET ligands for measuring the density of vesicular monoamine transporter (VMAT2).

These radiotracers are markers of dopamine storage capacity, vesicular monoamine and dopamine transporter availability. While dopaminergic neuronal loss leads to motor disability, non-dopaminergic neurotransmitter dysfunction is implicated in non-motor symptoms including sleep disturbance, fatigue, depression, dementia, and autonomic dysfunction. PET and SPECT utilizing different ligands, than those used to interrogate dopaminergic transmission, exist and can be used to evaluate the function of monoaminergic and cholinergic circuits in the brain.

Several other imaging biomarkers using other ligands such as [18F] deoxygluose for mitochondrial bioenergetics, [18F]BMS for mitochondrial complex-1, [11C](R)-PK11195 for microglial activation, also exist [202].

The central pathology in PD however revolves around alpha-synculein and currently no imaging bio-marker exists to visualize alpha-synuclein aggregates in neurons compared the Pittsburgh binding agent for amyloid aggregation in Alzheimer's disease. New frontiers in PD imaging to fill this void may evolve over the next few years to decades.

iv. Biochemical biomarkers

Several research groups have investigated a variety of clinical, imaging or biochemical assays, alone or in combination, to find a clinically useful biomarker of PD. It has been argued that perhaps the most promising is the assay of alpha-synuclein in the diagnosis and its accumulation in the prognosis of Parkinson disease. At present, detection protocols are still being refined, so that this is a process in evolution [203].

We conducted a systematic review of alpha-synuclein in body's tissues excluding the central nervous system and bio-fluids to investigate its utility as a potential biomarker of PD.

Alpha-synuclein in peripheral tissues and body fluids as a biomarker for Parkinson's disease - a systematic review.

Abstract

Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder that affects the elderly, with a prevalence of approximately 1% among those older than 65 years. PD is neuropathologically characterized as an alpha-synucleinopathy. Alpha-synuclein aggregates accumulate in Lewy bodies, which are the pathological hallmark of PD. However, alpha-synuclein containing inclusions in PD are not restricted to the central nervous system, but can also be stained in several peripheral tissues and alpha-synuclein monomers and oligomers are detected in various body fluids.

Objective

To conduct a systematic review of available evidence for the utility of alpha-synuclein as a peripheral biomarker of PD.

Methods

Search strategy

PUBMED (1948 to May 26, 2013), Embase (1974- May 26, 2013), the Cochrane Library (up to May 26, 2013), LILACS (up to May 26, 2013) and CINAHL (up to May 26, 2013) were searched for studies of alpha-synuclein in peripheral tissues or body fluids in PD patients.

Selection criteria Reports containing more than 5 subjects (patients and controls) were included where PD was diagnosed during life using predefined criteria, and the study found evidence of alpha-synuclein in peripheral tissues or body fluids.

Data analysis

Sensitivity and specificity of individual tests were calculated comparing PD cases with healthy controls, and did not include cases with incidental Lewy body disease.

Results

A total of 49 studies fulfilled the search criteria. Peripheral tissues such as colonic mucosa showed a sensitivity of 42-90% and a specificity of 100% ; labial salivary glands showed 66% sensitivity and 100% specificity; submandibular salivary glands showed sensitivity and specificity of 100%; skin biopsy showed 19% sensitivity and 80% specificity in detecting alpha-synuclein pathology. CSF alpha-synuclein had 71-94% sensitivity and 25-53% specificity for distinguishing PD from controls. Plasma alpha-synuclein had 48-53% sensitivity and 69-85% specificity in sporadic PD. Variability was at least in part methodological (e.g. assay techniques included enzyme linked immunosorbent assay (ELISA), Western blots and Luminex assays).

Conclusion

Alpha-synuclein is often present in peripheral tissues in PD, but tissues with the highest sensitivity and specificity (e.g. vagus and sympathetic ganglia) are difficult to access for biopsy. Endoscopic gastrointestinal biopsy may offer a relatively less invasive option while maintaining high sensitivity. However the confounding factor of incidental Lewy body disease and the non-specificity of alpha-synuclein to PD remain major obstacles in test interpretation. Body fluids are less reliable than solid tissue samples for differentiating alpha-synucleinopathies from other diseases or normal controls.

2.1 Introduction

The synucleins include alpha-, beta- and gamma-synuclein, and are a family of small, soluble proteins expressed primarily in neurons, but also found in lower concentrations in other tissues. Alpha-synuclein was first identified in human brain tissue during the ultrastructural study of amyloid plaques in patients with Alzheimer's disease in the early 1990's. Detailed analysis of the amino acid sequence in an amyloid preparation revealed a second component, in addition to the major A-beta fragment that was already known. It was called NAC (non A-beta component) and its precursor was named NACP (NAC precursor) [204]. A computer homology search established that the human *NACP* gene, which codes for alpha-synuclein, was homologous to the rat synuclein gene and mapped to chromosome 4q21 [205]. The human *NACP* gene was therefore redesignated as *SNCA.*

2.1.1 Structure of alpha-synuclein

Alpha-synuclein is formed of a varying number of amino acids as a result of alternative splicing of the *SNCA* gene. Alpha-synuclein 140 represents the whole transcript and is the major variant. Shorter forms have 126, 112, or 98 amino acids[206]. The 140 variant retains all the sites that undergo post-translational protein modification, while the shorter forms do not, and may be at the greatest risk of abnormal aggregation. Alpha-synuclein 140 has an N-terminal helix, a central helix and an unorganized, negatively charged C-terminal [206]. The N-terminal helix is characterized by lipid affinity that anchors alpha-synuclein to membranes and assembles lipoprotein complexes, while the hydrophobic central region is prone to intermolecular interactions, which may promote the aggregation of soluble alpha-synuclein monomers into insoluble oligomers and polymers[207]. Deletion or disruption of this region blocks this abnormal aggregation. The C-terminal charge qualitatively influences the kinetics of alpha-synuclein aggregation. The higher its content in negative amino acid residues, the lower the aggregation rate of alpha-synuclein thereby countering the pro-aggregatory potential of the central region[208].

2.1.2 Physiological role of alpha-synuclein

Alpha-synuclein accounts for as much as 1% of total protein in soluble cytosolic brain fractions, suggesting a significant role in neuronal function [209]. Physiologically the normal protein modulates the stability of the neuronal membrane, and it may also integrate presynaptic signalling and membrane trafficking via vesicular transport [210]. In experimental physiological conditions an extended monomeric conformation is its intrinsic state, but extreme environmental sensitivity causes alternative conformations, both physiological (e.g. monomeric alpha-helix and beta-sheet rich species, and dimers) and pathological (e.g. oligomers, polymerization into fibrils), the latter developing into the cytoplasmic inclusions (Lewy neurites and Lewy bodies) that mark disease states [211].

2.1.3 Mechanisms of abnormal alpha-synuclein aggregation

Alpha-synuclein belongs to the intrinsically disordered protein class. Environmental agents such as neurotoxins and genetic mutations may induce alpha-synuclein misfolding [212]. Alternative splicing of the *SNCA* gene may result in variations in protein structure, altering its aggregating propensity. Further protein modification after mRNA translation can occur by phosphorylation, ubiquitination, nitration, and truncation. All of these changes may result in oligomer formation and accumulation [206]. The mechanisms proposed to describe the neurotoxicity of alpha-synuclein and its aggregates include mechanical distortion of cellular compartments/processes, toxic gain of function, or loss of physiological function, and such mechanisms may be synergistic [213].

2.1.4 CNS distribution of alpha-synuclein aggregates

Alpha-synuclein inclusions are deposited in both dopaminergic and non-dopaminergic neurons, and in glia [207]. In PD and dementia with Lewy bodies (DLB), these inclusions are present both in cell bodies and the axonal processes of neurons and glia, as two morphologically distinct entities: Lewy bodies and Lewy neurites. In multiple system atrophy (MSA), the inclusions are seen as glial cytoplasmic inclusions (GCI) and in the neuronal cytoplasm. In neuroaxonal dystrophy (also called neuronal brain iron accumulation type 2), the inclusions are present as axonal spheroids [214].

2.1.5 Lewy bodies, Lewy neurites and alpha-synuclein in PD

Aggregated alpha-synuclein is the major component of Lewy bodies, the neuropathological hallmark of PD [215]. Besides the substantia nigra in the midbrain, Lewy bodies also are found in other brain regions, such as the dorsal motor nucleus of the vagus, the nucleus basalis of Meynert, the locus coeruleus and more diffusely in the brain in the later stages of PD [215]. Lewy bodies have a diameter of 5 to 25 microns, and a dense eosinophilic core with a surrounding halo [216]. Lewy neurites contain filaments similar to those of Lewy bodies [217]. Point mutations and multiplications in the *SNCA* gene cause autosomal dominant PD, further supporting the pathogenic role of alpha-synuclein in PD [6]. However, aggregation of alpha-synuclein is also a marker of other neurodegenerative diseases (the synucleinopathies) including DLB, the Lewy body variant of Alzheimer's disease, MSA, and neurodegeneration with brain iron accumulation type 1 [207].

2.1.6 Measurement of alpha-synuclein and detection of Lewy bodies

Alpha-synuclein is also present in red blood cells, cerebrospinal fluid, plasma and saliva, in both monomeric and oligomeric forms, challenging previous concepts that it may be a purely intracellular protein [218, 219]. Measurement of both monomeric and oligomeric forms in body fluids uses techniques such as ELISA or Western blots. Lewy bodies can be visualized in neurons of the central and autonomic nervous system from biopsies taken from various tissues including the gastrointestinal submucosa and salivary glands. Traditional staining shows these to be eosinophilic, but alpha-synuclein immunohistochemistry (antibodies to the amino- and carboxyl-terminal sequences) is now the standard method of localizing Lewy bodies and Lewy neurites in tissue specimens [220]. Proteomic identification by comparative mass spectrometry has identified around 40 other proteins including several kinases and ligases that may be involved in alpha-synuclein aggregation in the Lewy bodies [221].

2.1.7 Distribution of alpha-synuclein in Parkinson’s disease

The molecular basis of PD is closely linked to the abnormal aggregation of alpha-synuclein and factors affecting its conformation [217]. A pre-symptomatic phase of PD is well recognized, involving neurodegeneration in the substantia nigra

progressing to a critical threshold [222] but extra-cerebral pathology accompanies or even predates this, raising the possibility of pre-symptomatic detection from peripheral tissues [223].

2.1.8 Distribution of alpha-synuclein in the healthy elderly

Lewy bodies are present in autopsy brain specimens in 10 to 12% of people aged over 60 years without clinical parkinsonism [224], raising a potential problem for the interpretation of alpha-synuclein as a marker of pre-symptomatic or early PD. An alternative interpretation is that such Lewy bodies reflect age-related change in alpha-synuclein, without clinical significance to the development of PD, sometimes referred to as incidental Lewy body disease (iLBD) [225].
Alpha-synuclein also aggregates in a wider group of neurodegenerative conditions, such as DLB and MSA, and we excluded studies that were restricted to those other diseases.

2.2 Methods

2.2.1 Criteria for considering studies for this review

i. Types of studies

A search for all studies of alpha-synuclein inclusions in tissues other than the brain or spinal cord, or monomers/oligomers of alpha-synuclein in body fluids was performed. The material sampled could include ante-mortem biopsy tissue, body fluid or autopsy tissue specimen. Studies limited to alpha-synuclein pathology only in the central nervous system, review articles, letters in reply to another original article (unless presenting original data), abstracts without full articles, and studies without controls were excluded.

ii. Types of participants

We included reports where tissue samples were collected either during life or post-mortem in 5 or more subjects (clinically diagnosed PD patients with controls). Patients were included

provided their diagnosis was made by an experienced movement disorder physician, such that history, examination and investigation excluded alternative diagnoses.

iii. Types of outcome measure

The main aim was to review alpha-synuclein as an *in vivo* marker of PD, from accessible peripheral specimens (fluids and tissues). The primary outcome measure was the proportion of patients diagnosed clinically as PD, who had a tissue level of alpha-synuclein (detected by various immunochemical methods) that was statistically different from controls.

2.2.2 Search methods for identification of studies

Ovid databases (PUBMED and Embase) were searched combining medical sub headings [MeSH] Parkinson* disease and alpha-synuclein combined with the boolean operator AND, limiting the searches to humans and English language. Duplicate searches were then removed. Separate searches were conducted in CINAHL, the Cochrane library and LILACS using the same strategy. References in all relevant papers were hand searched for additional publications.

2.2.3 Data collection

Abstract matching to the selection criteria was performed independently by 2 authors (NM,DS) and resolution of differences by a third author (KG). 6281 abstracts were identified in Ovid databases Medline and Embase, 232 from CINAHL, 3 abstracts in the Cochrane library and 3 abstracts in LILACS. Limiting to human studies removed 1951 abstracts and restricting to English language removed a further 182; removing duplicates excluded a further 148 abstracts. Additional hand searching from references identified provided a further 20 abstracts. Of the remaining 4020 abstracts, 49 fulfilled the criteria of 5 or more PD patients and/or controls, with information available to assess for alpha-synuclein levels in peripheral body fluids or tissues. (Figure 2.2)

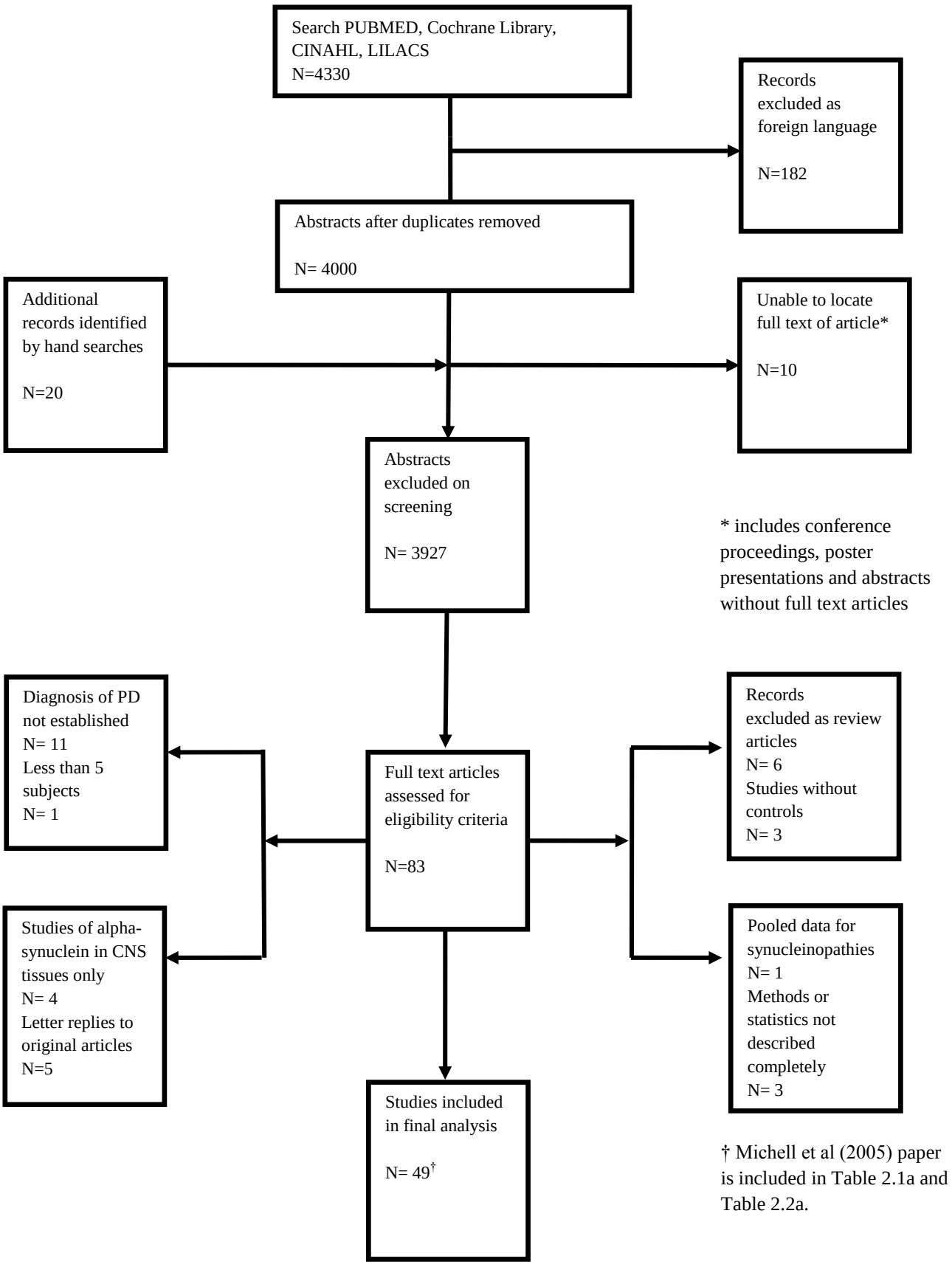

Figure 2.2 PRISMA diagram showing the process of paper selection for this systematic review.

2.3 Results

The results of all the studies that fulfilled the inclusion criteria for this systematic review are tabulated in **Table 2.1a and 2.1 b** for solid tissues and in **Table 2.2a and 2.2b** for body fluids in chronological order with calculations of sensitivity and specificity where available.

Table 2.1a Summary of 20 studies of alpha-synuclein pathology in solid tissues outside the central nervous system in humans.

Authors (year)	**Specimen (type of study)**	**PD (n)**	**Controls (n)**	**iLBD (n)**	**Methods**	**Alpha-synuclein positive cases/total**	**Sensitivity (%)**	**Specificity (%) PD versus Controls**
Iwanaga et al (1999) [226]	Cardiac plexus, sympathetic ganglia (post-mortem)	11	25	7	IHC	Positive in PD 9/11, iLBD 7/7 PD 9/11, iLBD 5/7	PD 88, 71-100 iLBD	100
Duda et al (1999) [227]	Olfactory mucosa (post-mortem)	5	11	NA	IHC	PD 5/5, Controls 11/11	100	0
Michell et al (2005) [228]	Skin (ante-mortem)	16	5	NA	IHC	PD 3/16 Controls 1/5	19	80
Braak et al (2006) [223]	Gastric myenteric plexus (post-mortem)	3	5	2	IHC	PD 3/3 iLBD 2/2 Controls 0/5	PD 100 iLBD 100	100
Fujishiro et al (2008) [229]	Heart (post-mortem)	14	4	11	IHC	PD 14/14 iLBD 7/11	PD 100 iLBD 63	100
Lebouvier et al (2008) [230]	Colonic tissue (ante-mortem)	5	5	NA	IHC	PD 4/5 Controls 0/5	80	100
Orimo et al (2008)[231]	Cardiac sympathetic nervous system (post-mortem)	10	10	20	IHC	PD 6/10 iLBD 18/20 Controls 0/10	PD 60 iLBD 90	100
Orimo et al (2008) [232]	Cardiac sympathetic nervous system (post-mortem)	3[a]	3	0	IHC	PD 3/3 Controls 0/3	100	100
Beach et al (2009) [233]	Olfactory mucosa (post-mortem)	58	69	21	IHC	PD 55/58 iLBD 14/21 Controls 5/69	PD 95 iLBD 67	91
Ghebermedhin et al (2009) [234]	Heart [b] (post-mortem)	5	2	7	IHC	PD 3/5 iLBD 2/2	PD 60 iLBD 100	100
Witt et al (2009) [235]	Olfactory mucosa (ante-mortem)	7	25	NA	IHC	PD 0/7	0	NA

PD=Parkinson's disease; iLBD=incidental Lewy Body disease; IHC=immunohistochemistry; NA=not applicable or not available; [a] PD patients with SNCA duplication, [b] epicardium, myocardium and conduction system, [c] larynx, primary bronchus, lung

Table 2.1b is a continuation of Table 2.1a

Authors (year)	**Specimen (type of study)**	**PD (n)**	**Controls (n)**	**iLBD (n)**	**Methods**	**Alpha-synuclein positive cases/total (p value)**	**Sensitivity (%)**	**Specificity (%) PD versus Controls**
Beach et al (2010) [236]	Multiple body sites (post-mortem)	17	23	7	IHC	PD (12/15 sympathetic ganglia, 11/15 vagus, 11/17 gastrointestinal tract, 1/8 respiratory tract [c], 2/9 endocrine system [d], 1/8 genitourinary tract).	PD (sympathetic ganglia 80;vagus 73; gastrointestinal tract 65; respiratory tract 12; endocrine system 22; genitourinary tract 12)	NA
Del Tredeci et al (2010) [237]	Submandibular salivary glands, autonomic ganglia, vagus (post-mortem)	9	19	3	IHC	Salivary glands: PD 9/9, iLBD 2/3, Controls 0/18 Superior cervical ganglion: PD 9/9, iLBD 2/3, Controls 0/8 Vagus: PD 9/9, iLBD 2/3, Controls 0/18	PD 100 iLBD 66 PD 100 iLBD 66 PD 100 iLBD 66	100 100 100
Lebouvier et al (2010) [238]	Colonic tissue (ante-mortem)	29	10	NA	IHC	PD 21/29	72	100
Cersosimo et al (2011) [239]	Labial salivary glands (ante-mortem)	3	3	NA	IHC	PD 2/3 Controls 0/3	66	100
Pouclet et al (2012) [240]	Rectal/ Colonic tissue (ante-mortem)	26	9	NA	IHC	PD (rectum 6/26, descending colon 11/26, ascending colon 17/26)	rectum 23, descending colon 42, ascending colon 65	100
Shannon et al (2012) [241]	Colonic tissue (ante-mortem)	10	23	NA	IHC	PD 9/10 Controls 0/23	90	100
Beach et al (2013) [242]	Sub-mandibular salivary gland (post-mortem)	28[e]	50	5	IHC	PD 28/28 iLBD 0/5 Controls 0/50	PD 100 iLBD 0	100
Mu et al (2013) [243]	Cervical part of vagus, pharyngeal plexus (post-mortem)	10	4	NA	IHC	PD 10/10 Controls 0/4	100	100
Pouclet et al (2012) [240]	Colonic submucosa (ante-mortem)	9	10	NA	IHC	PD 5/9 Controls 0/10	55	100

[d] adrenal, thyroid, parathyroid, testis and ovary ,[e] included 3 patients who met neuropathological criteria for PSP

Table 2.2a Summary of 30 studies of alpha-synuclein in body fluids in humans.

Authors (year)	**Specimen**	**PD (n)**	**Controls (n)**	**Methods**	**Alpha-synuclein PD versus controls (p value)**	**Sensitivity (%)**	**Specificity (%)**
Borghi et al (2000) [244]	CSF	12	10	IP and WB	NS	NA	NA
Li et al (2002) [245]	Platelets	13	11	WB	NS	NA	NA
El Agnaf et al (2006) [246]	Plasma	34	27	ELISA	Higher in PD (p=0.002)	53	85
Miller et al (2004) [247]	Blood	1	6	WB	Higher in PD (p<0.0001)	NA	NA
Michell et al (2005) [228]	Platelets	12	5	WB	NS	100	0
Tan et al (2005) [248]	Leukocytes	80	80	mRNA using PCR	NS	NA	NA
Lee et al (2006)[249]	Plasma	105	51	ELISA	Higher in PD (p<0.001)	NA	NA
Tokuda et al (2006) [250]	CSF	33	38	ELISA	Lower in PD (p<0.001)	NA	NA
Li et al (2007) [251]	Plasma	27	11	WB	Lower in PD (p=0.001)	NA	NA
Papachroni et al (2007) [252]	Plasma	31sPD 20 fPD	26	WB	Higher in fPD (p<0.001)	48 sPD 90 fPD	69
Fuchs et al (2008) [253]	Blood mononuclear cells	36	79	RTPCR ELISA	Related to genotype, see text	NA	NA
Ohrfelt et al (2009) [254]	CSF	15	55	ELISA	NS	NA	NA
Duran et al (2010) [255]	Plasma	95	60	ELISA	Higher in PD (p=0.0229)	NA	NA
Hong et al (2010) [b, c] [256]	CSF	117	132	Luminex assay	Lower in PD (p<0.001)	93†	39†
Shi et al (2010) [b, d] [257]	CSF	126	137	Luminex assay	Lower in PD (p<0.001)	92‡	38‡
Brighina et al (2010) [258]	Lympho-monocytes	78	78	ELISA	NS	NA	NA
Mata et al (2010) [259]	Plasma	86	78	Luminex assay	NS	NA	NA
Foulds et al (2011) [260]	Plasma	32	30	ELISA	NS	NA	NA
Parnetti et al (2011) [261]	CSF	38	32	ELISA	Lower in PD (p<0.05)	94	25
Yanamandra et al (2011) [262]	Plasma [a]	39 (EOPD 27 LOPD 12)	23	WB	Higher monomers in PD, (EOPD p<0.0001) (LOPD p<0.05)	NA	NA

PD=Parkinson's disease; CSF=cerebrospinal fluid; NA=not applicable or not available; IP=Immunoprecipitation; WB=Western blot; [a] antibodies to alpha-synuclein, ELISA=enzyme linked immunosorbent assay; mRNA=messenger RNA; NS= non-significant difference; PCR=polymerase chain reaction; sPD=sporadic PD; fPD=familial PD; RTPCR= real time polymerase chain reaction

Table 2.2b is a continuation of Table 2.2a

Authors (year)	**Specimen**	**PD (n)**	**Controls (n)**	**Methods**	**Alpha-synuclein PD versus controls (p value)**	**Sensitivity (%)**	**Specificity (%)**
Pchelina et al (2011) [263]	Leukocytes	8 LRRK2 33 sPD	18	WB	Lower in LRRK2 vs. sPD ($p<0.02$) and controls ($p<0.05$)	NA	NA
Mollenhauer et al (2011) [264]	CSF	257	47[e]	ELISA	Lower in PD ($p=0.0002$)	71	53
Devic et al (2011) [265]	Saliva	24	25	WB	NS	NA	NA
Park et al (2011) [266]	CSF, Plasma	23	29	ELISA	Higher CSF oligomers in PD ($p=0.039$) Total levels in CSF and plasma: NS	NA	NA
Foulds et al (2012) [f] [267]	CSF	39	20	ELISA	NS	NA	NA
Gorostidi et al (2012) [268]	Plasma	134 sPD 32 LRRK2	109	ELISA	Lower in sPD ($p=0.010$) NS in LRRK2	NA	NA
Hall et al [g] (2012) [269]	CSF	90	107	Luminex	Lower in PD ($p<0.01$)	NA	NA
Smith et al (2012) [270]	Serum	14	9	ELISA	NS	NA	NA
Besong-Agbo et al (2013) [271]	Serum	62	46	ELISA	Lower in PD ($p<0.05$)	NA	NA
Mollenhauer et al (2013) [272]	CSF	78	48	ELISA	Lower in PD ($p=0.002$)	NA	NA

[b] Data sets overlap ; [c] excluding cases with RBC contamination of CSF, [d] excluding cases with RBC contamination of CSF and age<50 years, EOPD=early onset PD; LOPD=late onset PD; LRRK2=leucine rich repeat kinase 2 positive; [e] normal controls, normal pressure hydrocephalus and progressive supranuclear palsy cases, [f] post-mortem collection of CSF, [g] excluding CSF samples with hemoglobin levels greater than 1000 ng/L

2.4 Discussion

A tissue biomarker for PD would be useful for diagnosis and disease monitoring, which might include assessing the effects of potentially neuroprotective therapy [273]. Such a biomarker might supplement or replace current screening approaches for early markers of pre-motor PD such as olfactory loss [172]. The attractiveness of alpha-synuclein as a potential biomarker for PD is clear from the large numbers of studies identified. Its pathological accumulation (whether intrinsically cytotoxic, or protective as a mechanism to eliminate damaged cellular components) is linked closely to the degenerative process [274, 275]. However, its presence in different forms, and within several tissues and fluids, adds complexity when evaluating it as a biomarker. In addition there are variations in case definition and disease duration within the studies reviewed, as well as the types of control cases included, and in the laboratory methods in detecting the various forms of alpha-synuclein. We will now discuss these various facets.

Firstly, the potential clinical setting of alpha-synuclein measurement varies between studies. As abnormal alpha-synuclein accumulation is a marker of a group of diseases called synucleinopathies, it should more readily distinguish PD from tauopathies such as progressive supranuclear palsy or Alzheimer's disease, but would not distinguish so easily from DLB or MSA, unless tissue distribution or levels in body fluids differed significantly. The inclusion of age-matched controls is important in such studies, since incidental Lewy bodies may be present with ageing and in the absence of a disease state, although the existence of preclinical PD is an added challenge of interpretation. Accordingly, we used normal controls (which were usually age and sex-matched) to determine sensitivity and specificity of tissue and fluid specimens in our analysis, and summarised but did not include data from cases defined as incidental Lewy body disease in these calculations. Secondly, studies that have been done so far are exclusively cross-sectional; monitoring disease progression by measuring peripheral alpha-synuclein levels in longitudinal studies has not been reported.

Compared to other potential biomarkers related to dopaminergic neurons, such as dopamine metabolites and dopamine transporters which are influenced by the dynamic responses to dopamine deficiency in the synaptic system and the use of dopamine

replacement therapies, alpha-synuclein may be considered as a more stable marker reflective of disease severity and progression, as it temporally and spatially aggregates in the brain with disease progression.

We first looked at the distribution of alpha-synuclein aggregates in peripheral tissues, in patients with PD, which could be obtained for diagnostic purposes and found that it is extensively distributed in several body tissues [236].While the presence of pathological alpha-synuclein in all peripheral tissues is included in this systematic review, information from tissues that are generally inaccessible to sample collection *in vivo,* but has been obtained from autopsy, is more relevant to the understanding of the pattern of distribution of alpha-synuclein in PD, and less relevant to its consideration as a practically useful biomarker. Therefore to aid in deciding the clinical usefulness of a tissue specimen for detecting alpha-synuclein pathology and the ease of obtaining the specimen for practical purposes we divided the results into those studies that required solid tissue from a biopsy/autopsy specimen and those analysing body fluids. Amongst solid tissue specimens the peripheral nervous system offered samples with the highest sensitivity, ranging from 73 to 100% for vagus nerve [236, 237], 60 to 88% for cardiac sympathetic ganglia [226, 231], 100% for superior cervical ganglion [237] and 100% for the submucosal nervous plexus in the stomach [223], but all studies were very small (between 3 and 15 cases). While specificity in these studies where healthy controls were included was 100% [223, 226, 231, 237], these studies also included cases of iLBD [223, 226, 231, 236, 237], which showed the presence of alpha-synuclein aggregates in a similar proportion of cases to PD [223, 226, 231, 237]. The presence of alpha-synuclein inclusions in these tissues is therefore not a reliably specific marker of PD; rather it is a marker of alpha-synuclein aggregation. While some consider iLBD to be a pre-morbid stage of PD [276], involving the concept of a threshold of Lewy body accumulation, an alternative interpretation is held by others, that such Lewy bodies reflect age-related change in alpha-synuclein, without clinical significance to disease development [225]. The detection of alpha-synuclein aggregates outside the brain (e.g. epicardial fat [277] , gastric tissue [278] and abdomino-pelvic autonomic plexuses in the general population [279])during life has extended the spectrum of incidental alpha-synuclein inclusions or immunoreactivity. While iLBD has traditionally been defined by post-mortem diagnosis from brain tissue

(on the basis of Lewy bodies in the substantia nigra and/or locus coeruleus without a clinical diagnosis of PD in life) [280], it does not cover the discovery of abnormal alpha-synuclein inclusions in peripheral tissues in living patients (in whom brain specimens have not been obtained). We therefore propose the term incidental alpha-synuclein aggregates (iAA) qualified by location: iAA-C for central nervous system such as the brain and spinal cord and iAA-P for alpha-synuclein aggregates in peripheral tissues. Whether iAA-P is pathological or not is entirely dependent on the interpretation of iAA-C, also called iLBD, as a preclinical disease state or an age-related phenomenon.

Peripheral nervous tissue showed high rates of detection of alpha-synuclein pathology in PD patients, but the practicality of obtaining some of the tissues studied (e.g. cardiac) is limited. Biopsy specimens from more accessible sites such as the colonic mucosa had moderate to high sensitivity (42-90%) and 100% specificity compared to healthy controls [230, 238, 240, 241]; submandibular and labial salivary glands also showed high sensitivity (66 to 100%) and specificity (100% versus normal controls), but again in very small studies (3 to 9 patients) [237, 239]. Biopsies of olfactory mucosa showed mixed results (sensitivity 0-100%, n=12) [227, 235]. Other tissues such as skin had low sensitivity in detecting alpha-synuclein pathology [228].

Alpha-synuclein is found normally in all blood cells including red blood cells, leukocytes, platelets, and body fluids including CSF, saliva and plasma [245, 248, 262, 265, 281]. The presence of alpha-synuclein in its various conformations in body fluids, compared to normal controls, therefore requires measuring concentration differences, to determine a cut-off value, in order to develop a diagnostic test or a biomarker for an alpha-synucleinopathy.

Conflicting results are reported for plasma alpha-synuclein levels. In 5 studies comparing *total* plasma alpha-synuclein between PD and controls, 2 reported elevated levels in PD [246, 249]; one found decreased levels in PD [251], and one found no significant difference [266]. Two studies reported no difference in *oligomeric* plasma alpha-synuclein (suggested as the neurotoxic species) [266, 282] while one study reported higher levels of antibodies to monomeric alpha-synuclein in PD [262]. There

was no difference in *phosphorylated* plasma alpha-synuclein (which is present in Lewy bodies) between PD cases and controls [260].

While the observation that alpha-synuclein may be actively secreted from degenerating neurons into CSF suggested potential utility as a biomarker of PD, results are no more consistent than for plasma. Decreased CSF alpha-synuclein levels in PD patients compared to controls [250, 256, 261, 264, 283], increased levels (of oligomeric alpha-synuclein) [264] and no significant difference [244, 254, 267] are all reported. For CSF alpha-synuclein, contamination from red blood cells which are a major source of alpha-synuclein [218, 281] requires consideration, but only 4 of 9 studies corrected for this factor. A further contributor to variation is reflected in a review of 242 studies up to 2005, which identified that only 75 studies (31%) used standardized commercial antibodies to alpha-synuclein, and dilution and antigen unmasking protocols varied between studies, even when the same antibody was employed [284].

None of the studies we reviewed examined alpha-synuclein as a marker of disease progression, which is consistent with negative findings from a systematic review of biomarkers relating to disease progression in PD [285].

2.5 Conclusion

It is clear that neither plasma nor CSF alpha-synuclein is presently a reliable marker of PD. This differs from alpha-synuclein in solid tissue samples of enteric and autonomic nervous system, which have potential as a surrogate marker of brain synucleinopathy, while recognising the important difference between such changes being incidental (or pre-clinical) or manifest. As such a single bio-marker, for example alpha-synuclein may not have clinical utility and it is probably the combination of a biochemical biomarker such as peripheral tissue alpha-synuclein, neuroimaging biomarker such as FP-CIT SPECT and clinical biomarkers such as olfactory dysfunction that may provide clues to diagnosing early PD. Clearly there is an unmet demand for better

tools that could be used as biomarkers that are practical and cost- effective in screening targeted populations.

Index terms

Medical Subject Headings (MeSH): Parkinson* disease; Alpha-synuclein
MeSH check words: Humans, English

Search Strategy

i. For Pubmed/Embase

1. parkinson*.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, sh, tn, dm, mf, dv, kw]
2. alpha-synuclein.mp. [mp=ti, ab, ot, nm, hw, kf, ps, rs, ui, sh, tn, dm, mf, dv, kw]
3. 1 and 2
4. limit 3 to "PubMed not Medline" [Limit not valid in Embase; records were retained]
5. limit 4 to humans
6. limit 5 to English language
7. remove duplicates from 6
8. limit 7 to full text

ii. For Cochrane Library using advanced search

MeSH parkinson* disease AND alpha-synuclein

iii. For CINAHL using advanced search

S1: alpha-synuclein AND parkinson* disease
Limiters - English Language; Exclude MEDLINE records; Human;

Search modes - Boolean/Phrase; Interface - EBSCO host .Search Screen - Advanced Search.
Database - CINAHL Plus with Full Text; PsycINFO

iv. For LILAC

Search Parkinson* disease AND alpha-synuclein
Limiters- English Language; Exclude Medline records; Human

Chapter 3. Clinical research protocol of PRoBaND

3.1 Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting the elderly and the numbers of people affected with PD, in countries of Western Europe, are projected to double by 2030 [286, 287]. This will have implications for the distribution of health care resources and more importantly it will be an economic burden on the patient and society [288]. Our understanding of the pathogenesis of PD, though still incomplete, changed significantly with the discovery of alpha-synuclein aggregation in Lewy bodies, the neuropathological hallmark of this disease [289]. Further confirmation of the central role of alpha-synuclein in the pathogenesis of PD was the finding of a specific mutation in the gene that codes for alpha-syunclein, *SNCA,* in some families with PD [6]. Mendelian genetics though, can only explain a small proportion of the total number of PD cases, and it is more likely that PD is a complex trait with both gene-gene and gene-environment interactions [290]. There is a need to find biomarkers that can help in diagnosing PD ,monitoring disease progression and determining response to therapy, given that we know that there is a pre-symptomatic phase where therapeutic interventions may have a better chance to affect the course of the disease [199].

3.2 Study design

The study is carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and is funded by Parkinson's UK. Ethics approval for the study was obtained from the West of Scotland multi-centre research ethics committee (reference 11/AL/0163, protocol 52504/1).

3.2.1 Study objectives

Primary objective:

To define the variation or heterogeneity in the clinical phenotypes of Parkinson's disease.

Secondary objectives:

i. To relate the variation in the clinical phenotype of PD to the underlying genotype of patients.
ii. To support related studies evaluating genetic, serum and imaging biomarkers for the diagnosis and progression of PD.

3.2.2 Study cohort

PRoBaND is the largest prospective, observational, multicentre study of PD in the United Kingdom (UK). We are recruiting 2000 patients with recent onset PD, defined as those diagnosed with PD in the last 3 years and 240 cases with early onset PD, defined as those with age at onset less than 50 years. We will also recruit 750 first degree unaffected relatives of patients with recent onset PD and 90 first degree relatives of cases with early onset who will serve as internal controls.

Inclusion criteria

A. Subjects with a diagnosis of PD

i. Diagnosis of PD, based on UK Brain Bank criteria and made within the preceding 3 years ('recent onset cases') or diagnosed at under 50 years ('under 50 years cases')
ii. Age $\geq$18 to $\leq$ 90years
iii. Subject is able and willing to provided informed consent.
iv. Subjects are allowed to enter the study after they have started anti-parkinsonian medication.

B. Subjects who are first degree (unaffected) relatives of recruited PD patients

i. Age ≥18 to ≤ 90years
ii. Resident in the United Kingdom and able to access one of the PRoBaND study centres.
iii. Subject is able and willing to provided informed consent.

Exclusion criteria

A. Subjects with a diagnosis of PD

i. Subject has severe comorbid illness that would prevent full study participation
ii. Subject has features indicating another type of degenerative parkinsonism, e.g. progressive supranuclear palsy
iii. Drug-induced parkinsonism (drug-unmasked PD is allowed)
iv. Symmetrical lower body parkinsonism attributable to significant cortical and/or subcortical cerebrovascular disease (patients with 'incidental' small vessel disease on brain imaging are allowed).
v. Negative or normal functional imaging of the presynaptic dopamine system
vi. The presence of UK Brain Bank exclusion criteria will be recorded at baseline, allowing for the presence of 1 or 2 exclusion criteria (e.g. dopamine antagonist Drug used; more than one affected relative) (if justified e.g. by abnormal SPECT).

B. Subjects who are first degree (unaffected) relatives of recruited PD patients

i. Subject has severe comorbid illness that would prevent study participation
ii. Subject already has a diagnosis of PD.

3.2.3 Recruitment sites

60 sites in the UK that provide standard care to PD patients as part of the National Health Service (NHS) have been selected for the PRoBaND study. Once approval has been obtained from the research and development (R & D) unit of the local health board and the local ethics

committee, the PRoBaND executive committee grants approval for site activation provided it is satisfied that a trained movement disorder specialist is involved in the provision of care of the PD patients and supervises the local staff recruiting subjects and administering study protocols. Unlike drug trials, there is no site initiation visit. But local principal investigator (PI) ensures research staff has undergone necessary training to ensure compliance with good ethical practice and NHS protocols. Verification of data acquisition, bio specimen collection and receipt across the multiple centres is reviewed on a weekly basis at the data co-ordinating centre, Glasgow, to ensure data validity.

3.2.4 Data acquisition, storage and validation

Data capture is by local medical and nursing staff, including PD nurse specialists where available at the various recruiting centres. Data recording is done directly on a secure and anonymised web-based electronic data capture system linked to the website www.clinbase.co.uk , but a paper case report form (CRF) is also available for centres unable to use the electronic-system. Missing and erroneous data points are pursued at the data co-ordinating center at Glasgow and information relayed to the study investigators to complete missing data points or rectify the erroneous data entries. Genetic data will be generated, analysed and stored at the central laboratory in Cardiff.

3.2.5 Clinical assessments

These are carried out at the local hospitals by trained medical staff where subjects have been recruited from and attend clinics for their routine clinical care. The study involves face to face interviews and clinical assessments recorded on standardised scoring sheets and validated scales to document the motor, non-motor, cognitive, behavioural features and therapy response of the enrolled subjects (Table 3.1). Blood samples are collected at baseline for DNA testing and serum samples are stored at -80^{o} centigrade in the central laboratory in Cardiff for proteomic analyses. Study follow up visits are spaced at 6 monthly intervals and for patient convenience are designed to overlap with their routine clinic visits (Figure 3.1)

Table 3.1 Clinimetric scales that are used during the study protocol (on the left) and biomarkers recorded or envisaged as add-ons to PRoBaND (on the right).

Clinical assessment	**Scale**		**Biomarker**	**Modality**
Motor	MDS-UPDRS		Imaging	CT, MRI, FP-CIT SPECT
Cognitive	MOCA		Serum	Alpha-synuclein
Autonomic	SCOPA-Aut Constipation questionnaire BP & HR recording		Genetic	*LRRK2, GBA, Parkin, PINK1*
Sleep	ESS, PSS			
RBD	RBD questionnaire			
NMS	NMSS			
Olfaction	UPSIT			
Neurobehavioral	HADS			
Personality	BFI			
Quality of Life	PDQ8 , EQ5D			
Past Medical and Family history	Structure questionnaires			
Environmental exposures	MERQ-PD-B			

MDS-UPDRS –Movement disorder society- Unified Parkinson's disease rating scale, CT- computed tomographic scan, MRI- magnetic resonance imaging, FP-CIT- SPECT- Iodine-123 fluoropropyl single photon emission computed tomographic scan, MOCA –Montreal cognitive assessment scale, SCOPA-Aut- Scales for Outcomes in PD- Autonomic, ESS- Epworth sleepiness scale, PSS- Parkinson's disease sleepiness scale, RBD- Rapid eye movement sleep behaviour disorder, NMSS- non-motor symptoms severity scale, UPSIT- University of Pennsylvania Identification test, HADS- Hospital Anxiety and depression scale, BFI- Big Five Inventory, PDQ 8-Parkinson's disease questionnaire 8 item, EQ5D- EuroQol questionnaire, MERQ PD-B- Mini Environmental Risk Questionnaire for Parkinson's disease patients baseline.

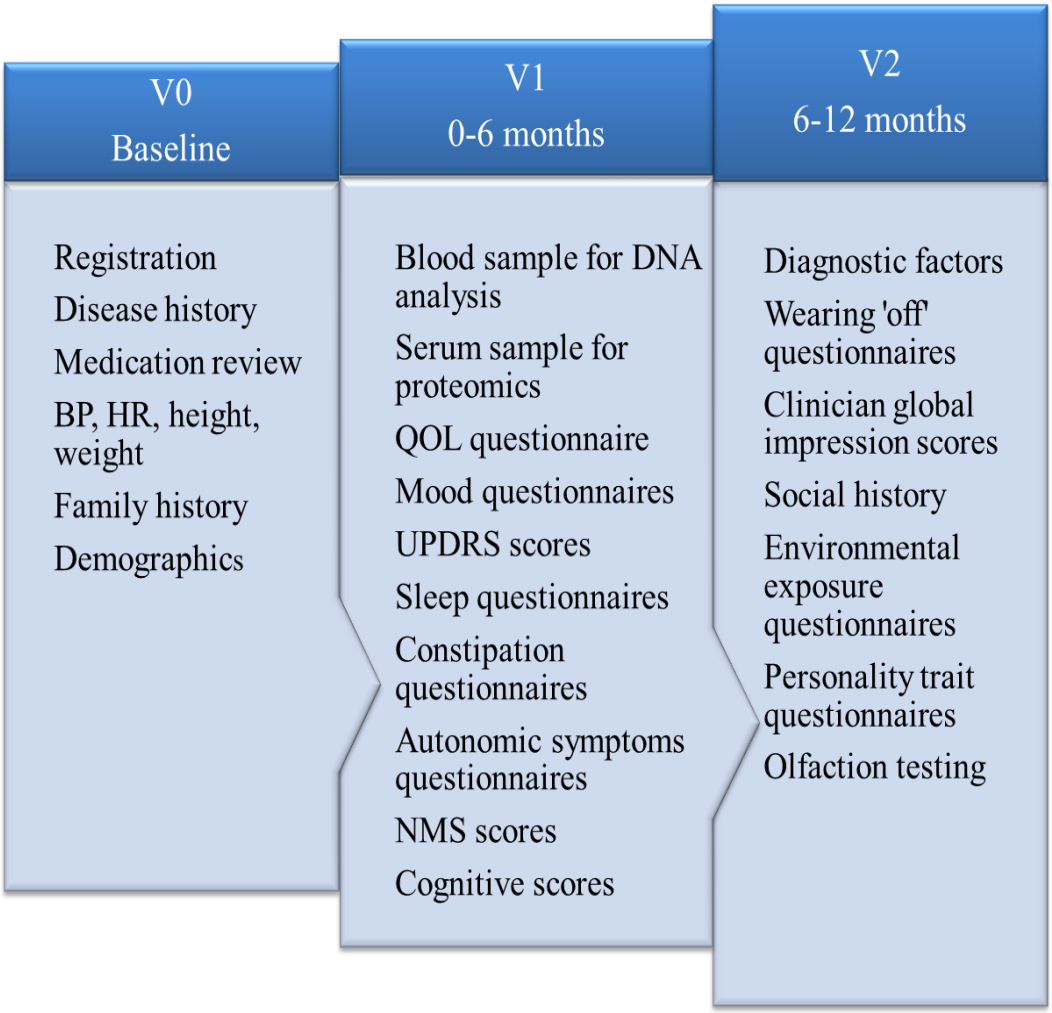

Figure 3.1 shows the timings of the visits and the assessments performed in the early onset Parkinson's disease (EOPD) arm of the study.

3.2.6 Blood sampling and genetic analysis

LRRK2 and *GBA* mutation carrier status will be tested in all PD patients. *Parkin* (PARK 2) mutations will be screened in early onset PD patients in the first phase with *PINK1* mutation screening later.

3.2.7 Proteomic analysis

Serum samples are collected at study entry (visit 0) for the early onset cases and stored for proteomic analyses at -80^{o} centigrade as described above. Alpha-synuclein has been investigated as a biomarker for PD [260] as have the isoforms of DJ1 protein [291]. These and other proteins that alpha-synuclein interacts with would be potential candidates for investigation as biochemical biomarkers. Using biomarkers to disentangle the different types of parkinsonism has been investigated [292] and there is a proteomic biomarker programme based in London and Oxford linked to our study.

3.2.8 Statistical analysis

The sample size for the bigger PRoBaND study has been calculated based on known incidence rates of PD and clinic activity levels in the NHS. This calculation was initially based on 24 recruiting sites. The number of centres has since increased, but we have left the target numbers unchanged, to allow for any delays in centre initiation and other contingencies. The study was designed to have a minimum of 240 patients in the EOPD arm of the study. Some patients who were recently diagnosed with PD also met eligibility criteria for inclusion in the EOPD arm. Inclusion of these additional patients (n=86) in the EOPD arm increased the sample size to 326. This was done assuming there would be data missing at the end of the study and those with >25% of questionnaires incomplete would be excluded from statistical analysis. 50 patients with incomplete datasets were therefore excluded, as no data imputation was allowed by protocol. This left us with datasets from 276 patients available for statistical analysis and the subsequent chapters in this thesis deal only with these patients with EOPD (Figure 3.2), except where indicated (Chapters 4 and 13). Consort diagrams, as an aid to explain how many case record forms were available prior to each statistical analysis, are shown at the beginning of the results section in each individal chapter from 4 to14 (Figures 4.1, 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 11.1, 12.1, 13.1, 14.1).

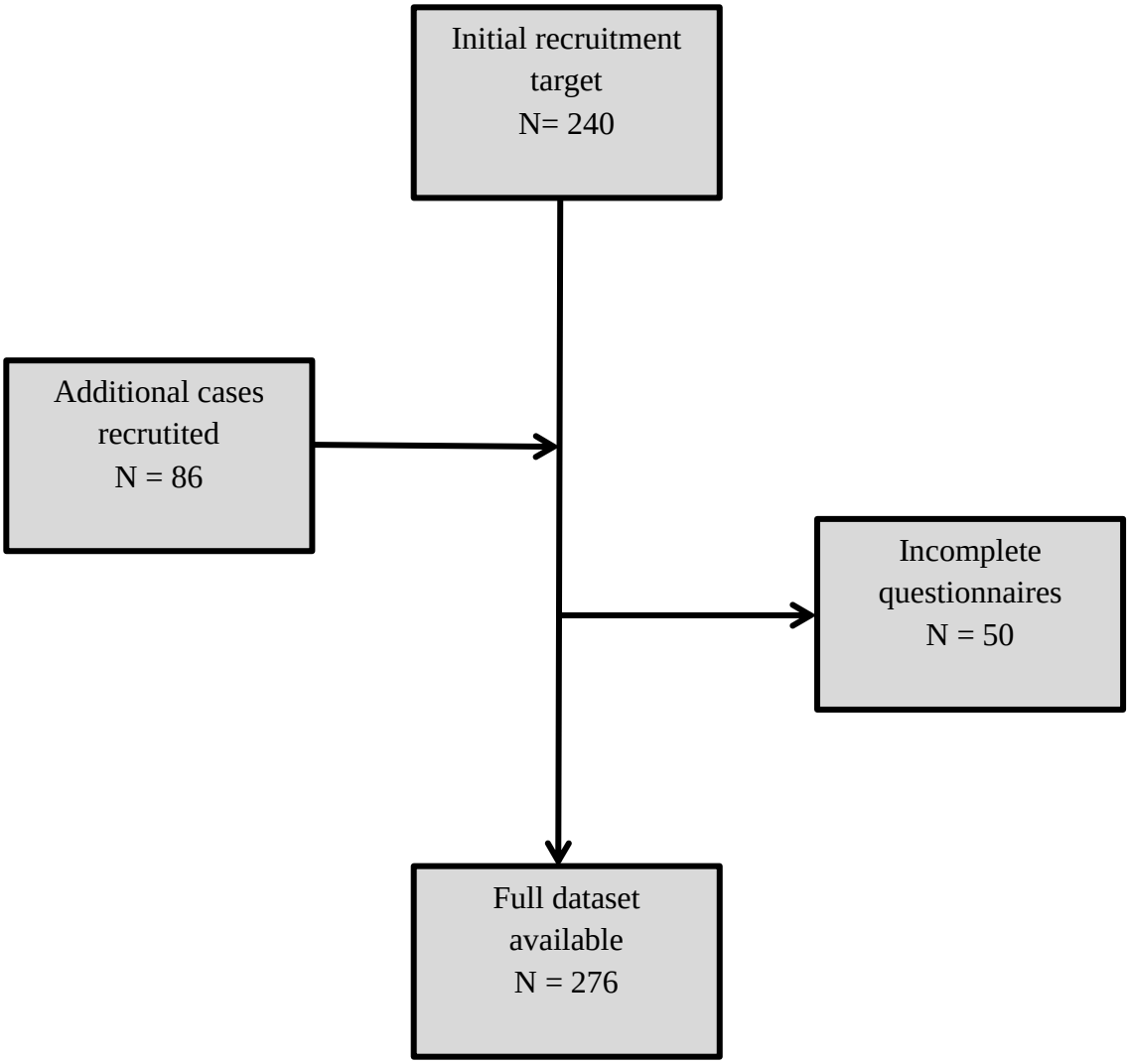

Figure 3.2 shows the disoposition of cases prior to data analysis. No data imputation was allowed. Those who had incomplete questionnaires were not included in data analysis.

Since PD is a multifaceted disorder, there are many possible ways to define subtypes. For example, PD subtypes may be based on motor features (e.g. tremor dominant vs. postural instability gait difficulty), age at diagnosis (less than 40 years vs. greater than 40 years), gender (males vs. females) and heritability of the parkinsonian trait (familial PD vs. sporadic PD). The onset of PD was defined, in line with previous studies as the time of diagnosis, not the retrospective report of first symptoms which is subject to a recall bias [293]. Familial PD was also defined in line with previous studies, as those cases showing a positive family history compatible with the diagnosis of Parkinsonism in at least one first or second degree relative [166]. Where a Mendelian pattern of inheritance in familial cases, either autosomal

dominant (across generations) or autosomal recessive (skipping generations but siblings may be affected), is obvious the term hereditary PD has been used [294]. While recognising that there may be several other ways of classifying EOPD patients e.g. by genotype, within the broader framework of this thesis, to analyse for the variation or heterogeneity in the clinical phenotype of EOPD patients with respect to historical and examination findings, all cases will be classified in 4 ways i.e. according to motor subtype, sex, age at diagnosis of PD and family history of PD.

In order to provide a uniform methodology, the computational models for statistical analysis and presentation of results (in tabulated form) using the '4 way classification' (Figure 3.3), described above, will be applied in all chapters of this thesis, except where specifically indicated.

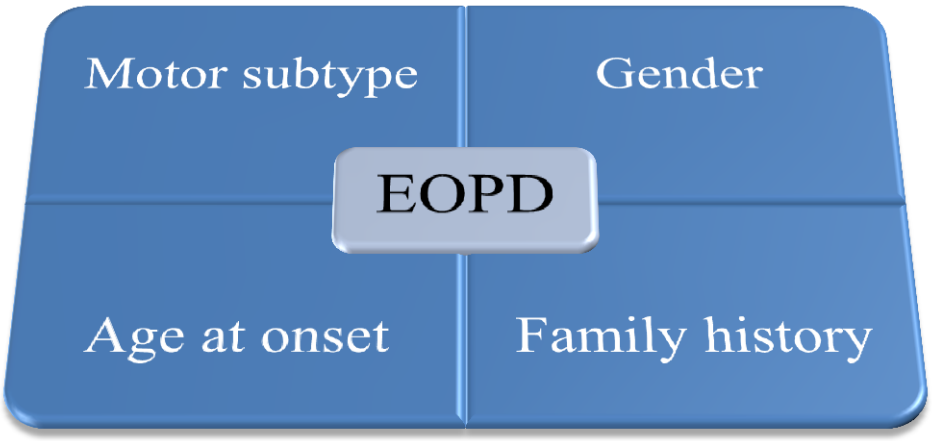

Figure 3.3 shows the 4 ways in which the early onset Parkinson's disease (EOPD) cases will be classified in all the chapters of this thesis, except where indicated.

3.2.9 Collaboration

Harmonization of datasets assists collaborative research. PRoBaND has adopted the common data elements (CDEs) of the National Institute for Neurological Diseases and Stroke (NINDS) [295]. The NINDS plans to enforce the use of CDEs in future US government funded PD research, which often includes international sites, e.g. the Parkinson Progression

Marker Initiative (PPMI) project. This will enhance compatibility and longevity of the datasets that will become available from the PRoBaND study.

In the UK we have linked up with Oxford Discovery Project, Cardiff Neurological Disease Bio bank and Neurogenetics Research Study (CANDAS), the NILS (Non-motor International Longitudinal Study) and the Brain Bank at Imperial College, London for tissue banking. (Figure 3.4)

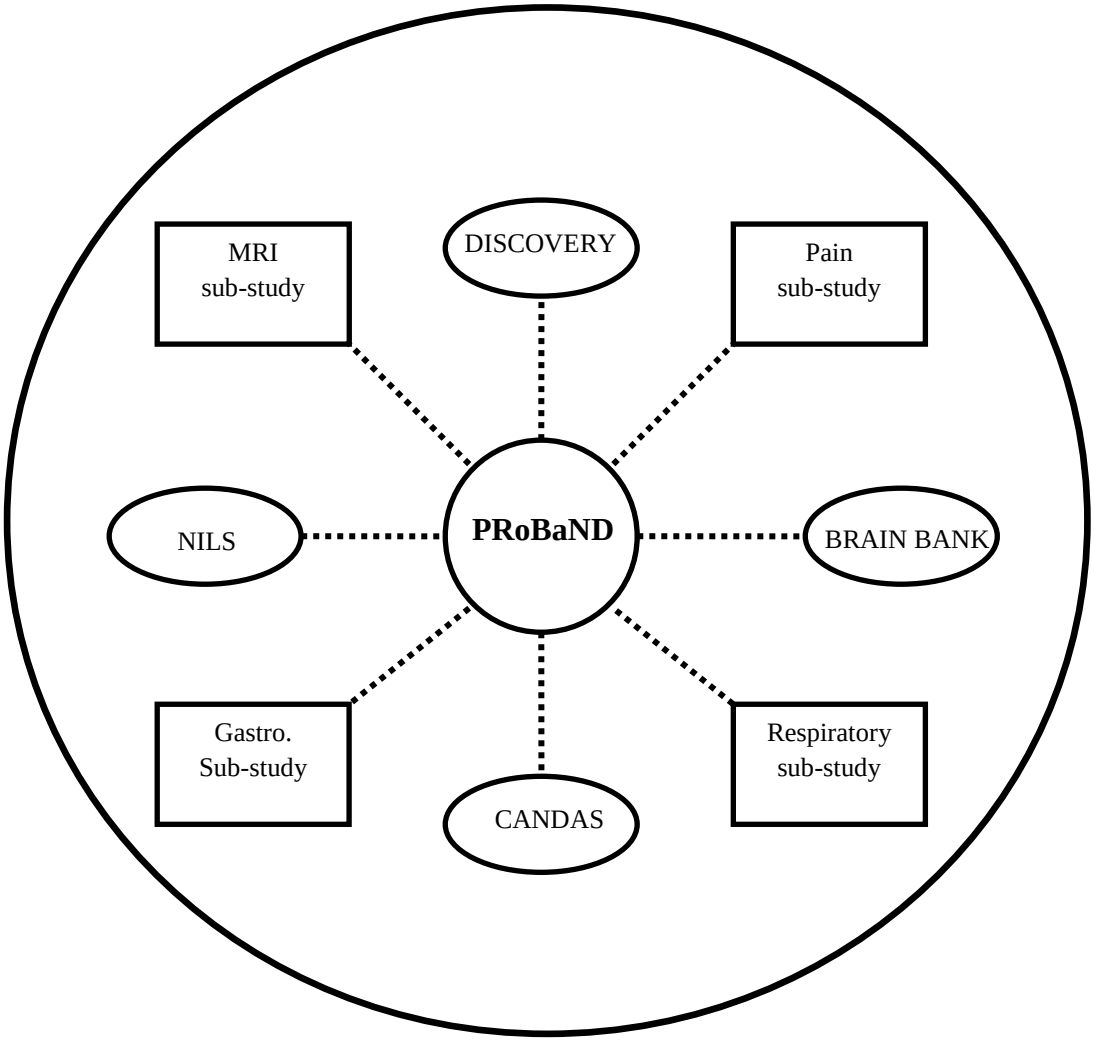

Figure 3.4 shows the 'hub and spoke' model of the PRoBaND study where sub-studies (rectangular boxes) link to the main study (circle) and collaboration with other similar studies (ovals) is supported, NILS=Non-motor international longitudinal study, CANDAS= Cardiff Neurological Disease Bio bank and Neurogenetics Research Study, BRAIN BANK= Imperial College, London, Brain Bank, DISCOVERY= Oxford Parkinson disease centre Discovery project.

3.2.10 Supporting future research

Data will be made available to support other research and audit projects in Parkinson's disease. Such data sharing will be stripped of personal identifiers, including date of birth and detail of family history. Access to such datasets will be on application to the executive committee chair. All studies seeking to use data and/or blood or serum samples will require ethics approval.

3.3 Discussion

PRoBaND seeks to establish a networked dataset of historical and clinical features of a large cohort of PD patients, establish patterns and variations in the phenotypes, acquire serum samples for proteomic analyses and collect DNA samples from all individuals to lay the groundwork for linking phenotypes with genotypes of these individuals. This is a process in evolution and will require a detailed analysis of the genomes of these individuals. Beyond traditional Sanger sequencing, genomic technologies have evolved over the last decade to include genome wide association studies (GWAS) which can indicate regions in the human genome that can confer the risk of disease and next generation sequencing ,such as exome sequencing, that can pinpoint novel genes that contain mutations [296]. In GWAS, the identification of genetic risk factors for the development of PD is achieved by analyzing as many as 500,000 different single-nucleotide polymorphisms (SNPs) in large groups of sporadic PD patients (a few thousands) and healthy individuals, and comparing SNP frequencies in the two groups. If certain variants are more frequent in PD patients, they are considered to be "associated" with the disease. These genetic variants are used to indicate the region of the human genome where the PD-causing change is likely to be situated.[297]

In the first phase we aim to test for Mendelian genes causing PD which will include *LRRK2* [298] *, GBA*[299] *and Parkin* [300] with *PINK1* [301] tested later, only in those with early onset disease.

Mendelian genes though account for less than 10% of the total cases of PD in the general population [302]. Mutations in the 4 genes that will be tested in the PRoBaND cohort are recognised as rare causes of PD but common variants in these genes and other related genes

may serve as susceptibility loci [303]. Single nucleotide polymorphisms (SNPs) at several genetic loci probably account for a substantial part of the variation in the clinical phenotypes of PD, however, a great deal of these common variants of small effect remain to be discovered [290]. In the second phase of our study we therefore aim to expand the use of genomic technologies to enhance our understanding of the genetic risks to individuals with PD. Sequencing for SNPs across the genome will serve as the goal of the second phase in the protocol. All DNA samples will be genotyped using the Illumina Human Core Exome array, supplemented with custom content. This will allow the analysis of approximately 250,000 common and 250,000 rare variants, plus over 27,000 custom variants selected because of their previous implication in a range of neurodegenerative, neurological, and psychiatric disorders [304, 305].

There will be a large number of SNPs identified in these loci and using public databases such as dbSNP we can filter the information to analyse which variants can influence the phenotypes comparing cases with controls [306].

The genetic basis of sporadic late onset PD is not clearly understood and this is likely to be a complex trait where genetic as well as environmental influences play a role through gene environment interactions although the molecular pathways for this have not been elucidated [307].

Finding a blood biomarker would be a very convenient way of diagnosing and recording progression of a disease process. Alpha-synuclein levels in the blood have been tested for use as a biomarker but the data so far have been less than convincing in establishing its utility for this purpose due the large variation in the results reported. However since alpha-synuclein is intimately linked to the pathogenesis of PD, either a specific confirmation of alpha-synuclein or a panel of plasma biomarkers that involves proteins that interact with alpha-synuclein would have to be investigated to determine whether this hypothesis is true or not. PRoBaND's 'hub and spoke' model (Figure 3.4) allows for this and a group based in London with expertise in tandem mass spectrometry and ELISA based protocols is in the process of commencing work on this.

Imaging biomarkers that are more economical and readily accessible to physicians than currently available modalities such as FP-CIT SPECT scans are needed. The nuclear

medicine scans currently in use are neither specific for PD, to differentiate it from atypical parkinsonism, nor can they be used to monitor disease progression or response to therapy. An imaging sub-study of PRoBaND is in place to start enrolling patients in one of the largest ever brain scanning studies in patients with PD. This will involve magnetic resonance image (MRI) scans of the brain at high resolution in 300 patients from the original PRoBaND cohort and an additional 100 people who will serve as normal controls. The data collected will be used to create a 'virtual brain bank' which will supplement the detailed clinical subtyping of PD that PRoBaND provides and this will be accessible to researchers all over the world to use as a platform for identifying novel imaging markers for PD.

Several other sub-studies linking with the hub include research teams looking at pain pathways, gastrointestinal dysfunction and respiratory disturbances in PD to provide an in-depth and comprehensive assessment of factors that could have an impact on the quality of life in PD.

Our study has similarities with other large cohort studies in PD such as the United States Parkinson's Progression Markers Initiative (PPMI) multicenter study (n=600) [308] , however, PRoBaND is a much larger multi-faceted study (n=3080) and is not limited to drug-naïve PD cases like PPMI but has a broader base and serves as a hub for several allied sub-studies in PD. So, while acknowledging some of our goals such as finding biomarkers for PD are shared with PPMI and other similar studies, PRoBaND offers a platform for researchers across the world to access datasets, DNA and bio samples of a large cohort of prospectively followed patients in order to better understand this disease and there is an unlimited scope for collaboration.

3.4 Conclusion

We have been successful in establishing a large effective clinical research network actively recruiting cases to a combined clinical-laboratory program, evaluating variation in the clinical expression of PD, which will be studied in relation to genetic influences, and offer a platform for finding serum and imaging biomarkers for this disorder. Collaboration with other groups in this scientific endeavour has been actively pursued in order to improve our understanding of PD and we anticipate that our ground work lays down the path for future research in the pathogenesis and treatment of this disease.

Chapter 4. Variation in premotor symptoms and other risk factors in EOPD

4.1 Objective

The objective in this chapter is to analyse the variation in the pre-motor characteristics and risk factors in a cohort of patients with early onset Parkinson's disease from the PRoBaND study.

4.2 Introduction

Parkinson's disease (PD) is recognised to have a pre-motor phase that may start several years, if not decades, before the classical motor features that are part of the diagnostic criteria[309] develop. Anxiety, depression, REM sleep behaviour disorder (RBD), olfactory loss and constipation have been considered pre-motor characteristics in PD[310]. Head injury and oophorectomy have been found to have an increased odds ratio of being associated with the subsequent development of PD in meta-analyses of epidemiological studies while smoking and caffeine consumption are reported to have a negative association with the future risk of developing PD [194, 311].

The study protocol of PRoBaND allowed for olfaction, constipation and RBD symptoms to be recorded using standard questionnaires prospectively, rather than retrospectively, hence the data on variation in these 3 symptoms have been presented in Chapter 7 which deals with all non-motor symptoms, rather than here.

4.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0); physical parameters such as weight, height and blood pressure were recorded at the same visit. Data required for Movement Disorder Society Unified Parkinson's disease rating scales (MDS-UPDRS) scores were collected at study visit 1 and a modified mini environmental risk questionnaire for Parkinson's disease patients' baseline (MERQ-PD-B) at study visit 2. This is one of the standard forms for collecting such information incorporating common data elements (CDE) suggested by the National Institute of Neurological Disorders and Stroke (NINDS). An overview of all PD CDE recommendations can be found in the PD CDE Highlight Summary document on their website [295].The MERQ-PD form was used to collect information for exposure to environmental risk factors such as head injury, consumption of caffeine and past history of oophorectomy in females. The MERQ-PD-B form was modified to include additional questions about pre-motor symptoms i.e. depression and anxiety prior to the diagnosis of PD. Head injury for the purposes of this study was defined as that causing either loss of consciousness or concussion as diagnosed by a doctor.

To analyse for the variation in clinical phenotype of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways as outlined in chapter 3. First patients were classified according to motor subtypes i.e. tremor dominant PD (TDPD), postural instability gait difficulty (PIGD) and 'Mixed' or 'Indeterminate', terms interchangeably used hereafter, subtypes based on their UPDRS scores according to a mathematical formula that incorporates MDS-UPDRS Part 2 (UPDRS2) and MDS-UPDRS Part 3 (UPDRS3) scores [312]. Tremor score is calculated as the mean score of the MDS-UPDRS elements describing tremor in the left column (0–4 for each item) of Table 4.1. PIGD score is calculated as the mean score for the posture, gait and instability while walking elements in the right column (0–4 for each item) of Table 4.1.

The ratio of the mean MDS-UPDRS tremor score (11 items, left column Table 4.1) to the mean UPDRS PIGD score (5 items, right column Table 4.1) was used to define TDPD motor subtype (ratio ≥1.5), PIGD motor subtype (ratio ≤1), and 'Mixed' or 'Indeterminate' motor

subtype (ratios >1.0 and <1.5). In addition, patients who had a positive mean in the numerator and a zero in the denominator were classified as TDPD; patients with a zero in the numerator and a positive mean in the denominator were classified as PIGD, and patients with zeroes in both the numerator and denominator were classified as 'Mixed'.

Table 4.1 Elements of the Movement Disorder Society Unified Parkinson's disease rating (MDS-UPDRS) scale used in calculating Tremor and PIGD scores.

Tremor score	**PIGD score**
MDS- UPDRS Part 2	*MDS- UPDRS Part 2*
1. Tremor	1. Walking and balance
	2. Freezing
MDS- UPDRS Part 3	*MDS- UPDRS Part 3*
2. Postural tremor RUE	3. Gait
3. Postural tremor LUE	4. Freezing of gait
4. Kinetic tremor RUE	5. Postural stability
5. Kinetic tremor LUE	
6. Rest tremor RUE	
7. Rest tremor LUE	
8. Rest tremor RLE	
9. Rest tremor LLE	
10. Rest tremor lip/jaw	
11. Rest constancy	

RUE= Right upper extremity, LUE=left upper extremity, RLE=Right lower extremity, LLE=Left lower extremity. Adapted from [312].

Further analyses looking at variation in the clinical phenotype involved 3 additional methods of subtyping cases: by gender, age at onset and heritability of the parkinsonian trait i.e. those with a positive family history of PD compared to those with no family history of PD.

Finally, considering that the most important risk factor for PD is a positive family history of PD [194] we analysed the hereditary patterns within the EOPD cohort by classifying patients into 2 groups, those who at age at onset of PD symptoms ≤ 40 years and those with age at onset of PD symptoms > 40 years to consider whether the cut-off of 40 years to define EOPD in other studies made a significant difference to the results from our study, where we have used 50 years as the cut-off to define EOPD.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes, with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data, with post-hoc tests as appropriate.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (version 4.0 for Windows; CA, USA) and SPSS (*version 23 for Windows*, IL, USA).

4.4 Results

210 patients had completed all questionnaires relevant to this analysis and are included here (Figure 4.1). The median age at the time of registration was 52.5 years (inter-quartile range, IQR, 47.4-56.6) and median disease duration was 7.5 years (IQR 3.7-11.5). Slightly more than half of the patients were males and the vast majority were Caucasians (Figure 4.2).

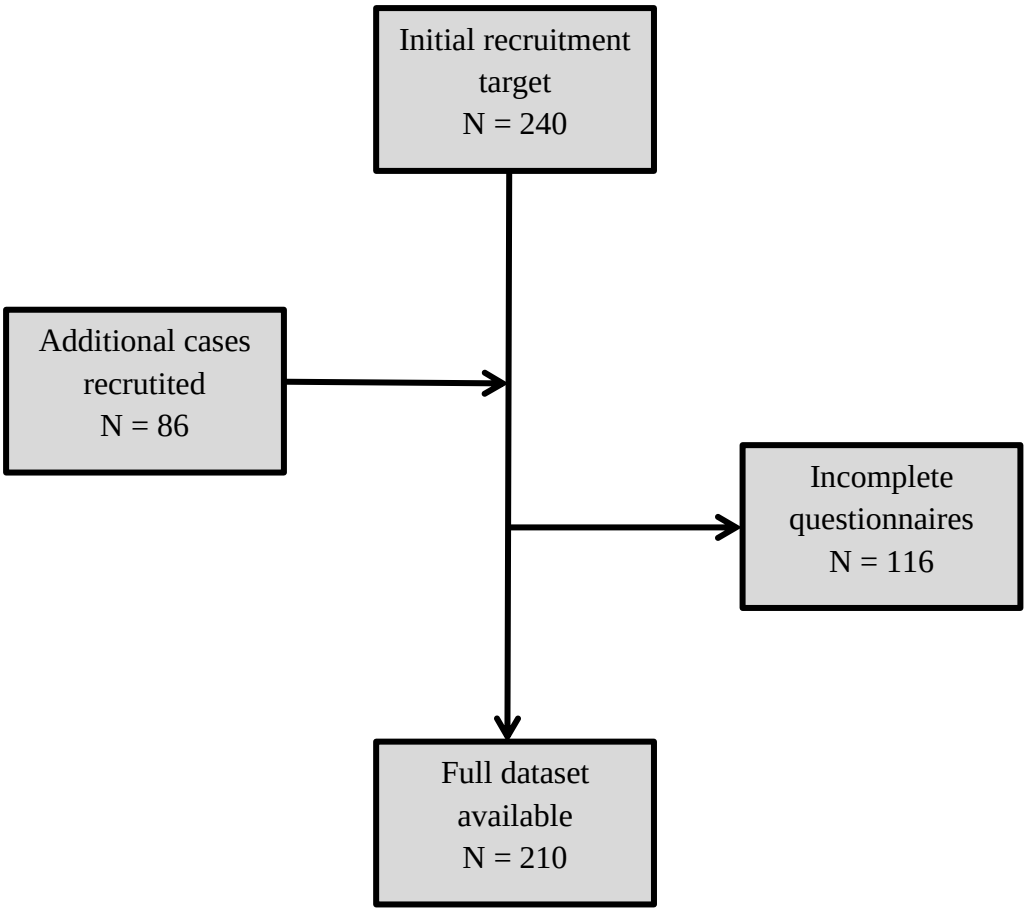

Figure 4.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

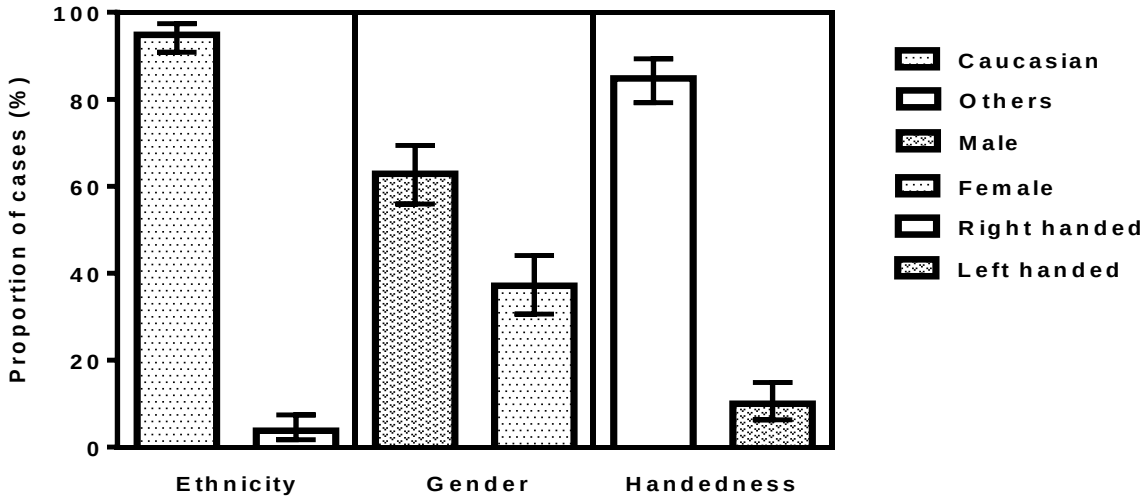

Figure 4.2 shows the demographic profile of the early onset PD cohort (n=210) used in this analysis, with proportion of cases on the y-axis and demographic characteristics on the x-axis.

Other demographic variables of these 210 patients are presented in Table 4.2. Those with missing data in the questionnaires analysed in this chapter (n=66) were not included and imputation of missing data was not used, in order to avoid drawing erroneous conclusions from the results of the statistical analysis.

Table 4.2 Demographic profile of the early onset PD patients (n=210) analysed in this section who had completed all relevant questionnaires.

Variable	**Median (Inter-quartile range)**
Number of cases	210
Age (at registration, in years)	52.5 (47.4-56.6)
Sex (males)	52.5%
Height (metres)	1.7 (1.7-1.8)
Weight (kg)	79.2 (65.6-95.2)
Handedness (right)	89.4%
Married	69.2%
Employed (at the time of diagnosis)	89.9%
Ethnicity	
Caucasian	94.8%
Asian	3.3%
African	0%
Caribbean	0.5%
Disease duration (years)	7.5 (3.7-11.5)

IQR= inter-quartile range. Data are presented as median (inter-quartile range) except where indicated

The variation in some of the important pre-motor characteristics and risk factors in patients classified by motor subtype is shown in Figures 4.3 and 4.4. There were 116 cases that were classified as TDPD, 79 cases were classified as PIGD and 15 cases were classified in the 'Mixed' motor subtype (Table 4.3).

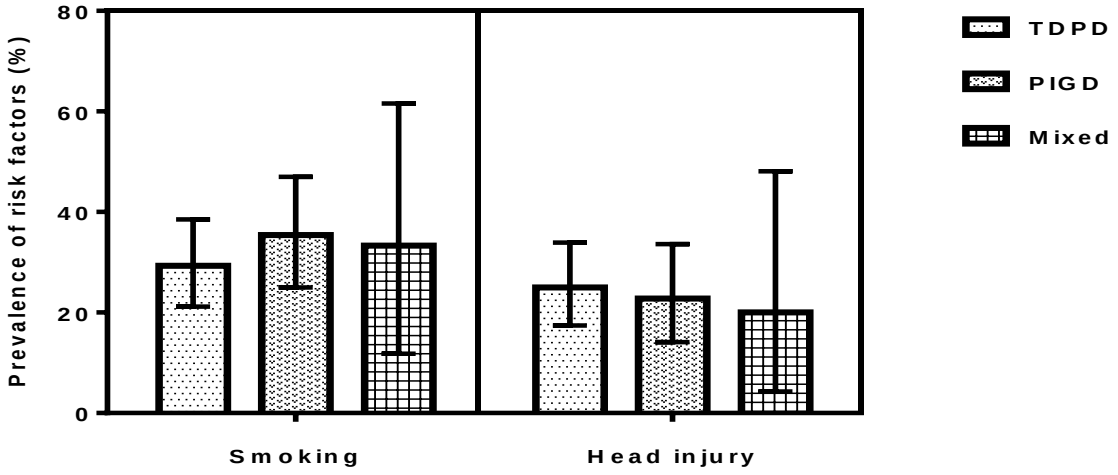

Figure 4.3 shows the prevalence of head injury and smoking, antedating the diagnosis of Parkinson's disease (PD), in the EOPD cohort of PRoBaND on the y-axis and the motor subtype of the patients on the x-axis. The top of the boxes represent the proportion of cases who were either smokers or reported head injury, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

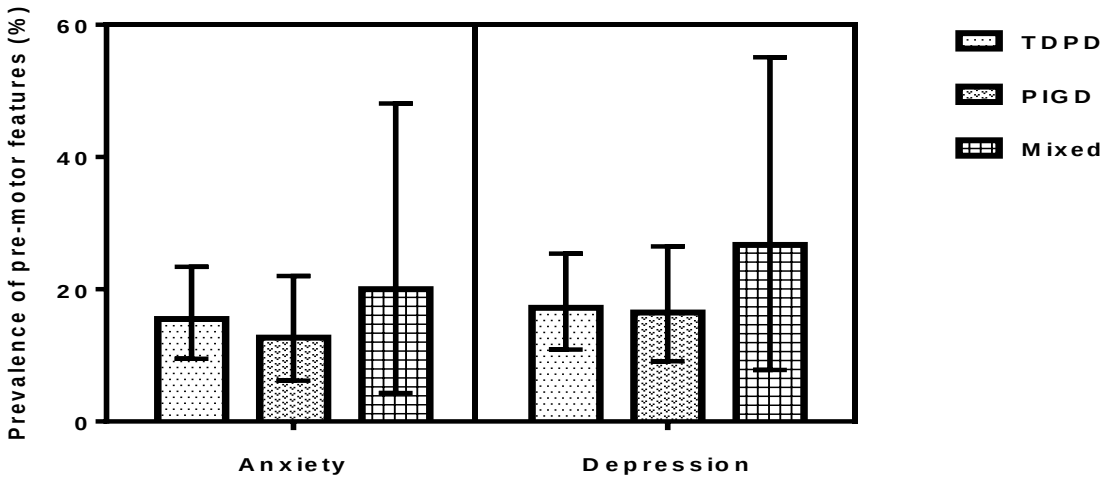

Figure 4.4 shows the prevalence of anxiety and depression, antedating the diagnosis of Parkinson's disease, as pre-motor characteristics in the EOPD cohort of PRoBaND on the y-axis and the motor subtype of the patients on the x-axis. The top of the boxes represent the proportion of cases who reported either anxiety or depresion, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

Patients in the PIGD were older (p<0.001) and had longer disease duration (p<0.001) compared to the other motor subtypes. Post hoc tests showed significant differences in age between TDPD and PIGD (p<0.001), PIGD and 'Mixed' (p=0.011) but were not significant between TDPD and 'Mixed' (p=0.993). The differences in disease duration on post hoc tests showed significant differences between TDPD and PIGD (p=<0.001) but not between PIGD and 'Mixed' (p=0.087) or between TDPD and 'Mixed' (p=0.303).

There were no statistically significant differences in the prevalence of pre-motor features such as depression and anxiety or risk factors such as head injury, caffeine intake and smoking in between cases classified by motor subtype (Table 4.3)

Table 4.3 Variation in pre-motor characteristics and risk factors for Parkinson's disease in patients (n=210) classified by motor subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**'Mixed'** median (IQR)	**p-value**
Number of cases	116	79	15	-
Age* (years)	51.2 (46.0-54.7)	55.0 (50.0-59.4)	51.2 (45.9-52.3)	**<0.001**
Sex (males)	65.5%	59.5%	60.0%	0.675
Duration† (years)	5.7 (2.9-10.1)	9.5 (5.4-15.1)	7.7 (3.9-11.2)	**<0.001**
Anxiety ‡	15.5%	12.7%	20.0%	0.719
Depression ‡	17.2%	16.5%	26.7%	0.628
Head injury ‡	25.0%	22.8%	20.0%	0.879
Caffeine ‡§	4.5 (3-8)	6 (3-8)	6 (4-6)	0.485
Smoking *	29.3%	35.4%	33.3%	0.661
Oophorectomy *	3.4%	0	0	NA

*** Age at registration, † disease duration, ‡ prior to diagnosis of PD, ‡§ coffee/tea cups per day.**

There were 132 males and 78 females in the analysed cohort (Table 4.4).Classifying patients by gender showed more males were exposed to cigarette smoke compared to females (p=0.046) but there were no statistically significant differences in other risk factors or pre-motor features analysed in between the two groups (Figures 4.5 and 4.6, Table 4.4)

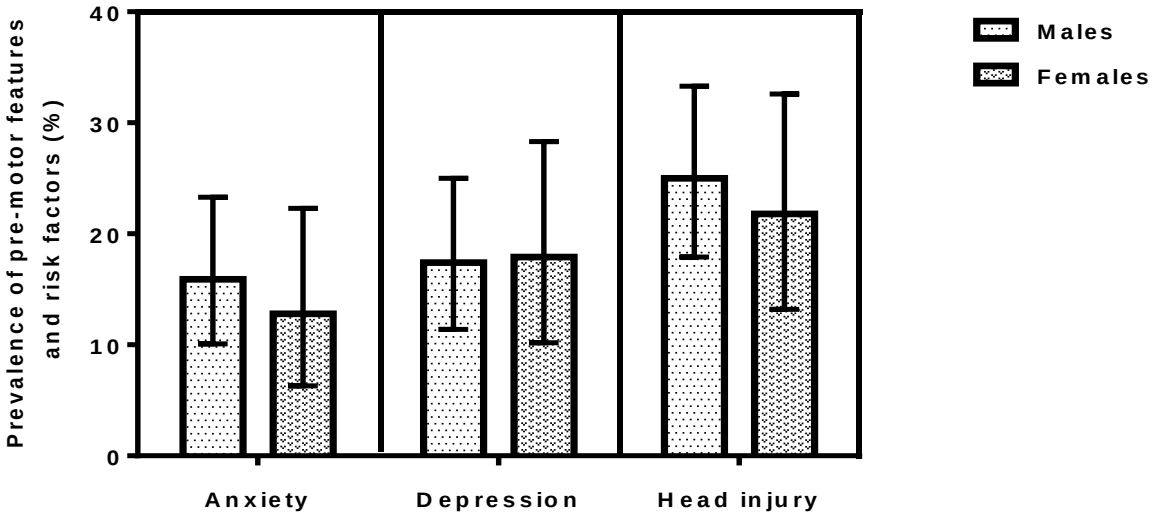

Figure 4.5 shows differences between males (on the left) and females (on the right) in the prevalence of pre-motor features and risk factors in the EOPD cohort of PRoBaND. The top of the boxes represent the proportion of cases who reported anxiety, depression or head injury antedating the diagnosis of Parkinson's disease, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

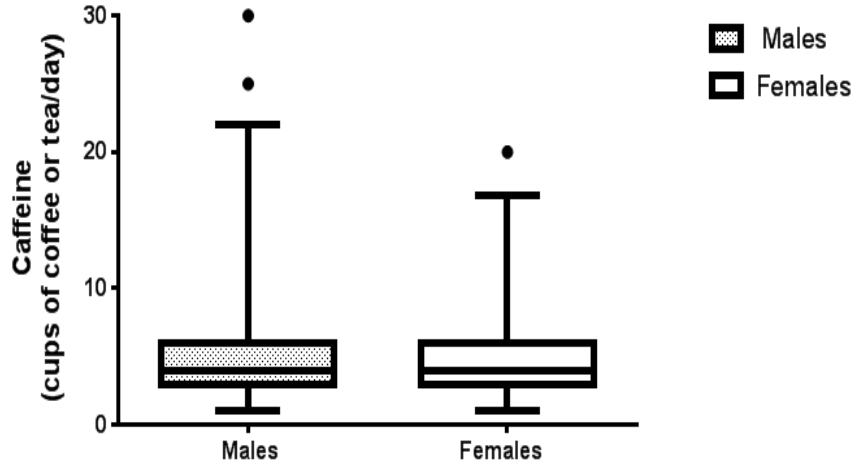

Figure 4.6 shows differences between males (on the left) and females (on the right) in caffeine intake (on y-axis) in the EOPD cohort (p=0.178) of PRoBaND. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles.

Table 4.4 Variation in pre-motor characteristics and risk factors in patients (n=210) classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p-value**
Number of cases	132	78	-
Age* (years)	52.5 (47.4-56.7)	52.2 (47.4-56.5)	0.906
Duration† (years)	7.7 (3.7-11.6)	6.7 (3.3-11.3)	0.362
Anxiety ‡	15.9%	12.8%	0.688
Depression ‡	17.4%	17.9%	1.000
Head injury ‡	25.0%	21.8%	0.620
Caffeine ‡§	5 (3-8)	5 (3.5-6.8)	0.178
Smoking ‡	37.1%	23.1%	**0.046**
Oophorectomy ‡	NA	2.6%	NA

*** Age at registration, † disease duration, ‡ prior to diagnosis of PD, ‡§ coffee/tea cups per day**

There were 50 cases that had been diagnosed before the age of 40 years and 160 cases that were diagnosed with PD after the age of 40 years in the EOPD cohort analysed (Table 4.5). Those diagnosed with PD before the age of 40 years had a longer disease duration (p=0.031) compared to those diagnosed after the age of 40 years in this cohort of EOPD patients but there were no statistically significant differences between any of the pre-motor features and risk factors analysed between the two groups (Figure 4.7 and Table 4.5).

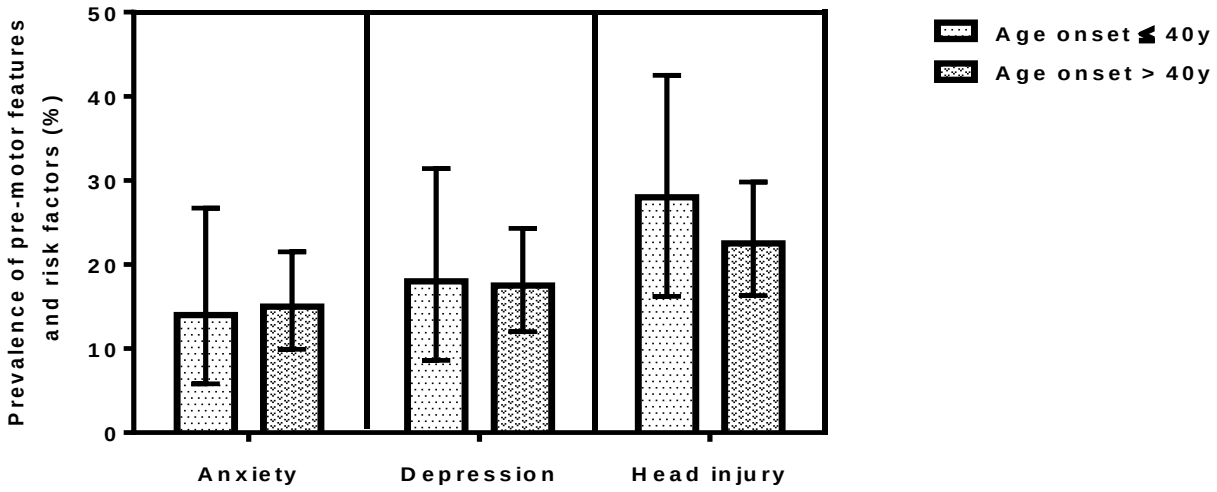

Figure 4.7 shows differences between those diagnosed with Parkinson's disease (PD) at or before the age of 40 years (on the left) and those diagnosed after the age of 40 years (on the right) in the prevalence of pre-motor features and risk factors in the EOPD cohort of PRoBaND. The top of the boxes represent the proportion of cases who reported anxiety, depression or head injury antedating the diagnosis of PD, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

Table 4.5 Variation in pre-motor characteristics and risk factors in patients (n=210) classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40 yrs.** median (IQR)	**p-value**
Number of cases	50	160	-
Age* (years)	47.2(41.6-51.9)	53.5(49.7-57.0)	**<0.001**
Sex (Males)	72%	60%	0.135
Duration† (years)	8.4(4.7-15.0)	7.2(3.1-11.2)	**0.031**
Anxiety ‡	14.0%	15.0%	1.000
Depression ‡	18.0%	17.5%	1.000
Head injury ‡	28.0%	22.5%	0.449
Caffeine ‡§	4.5(3-8)	5(3-8)	0.203
Smoking ‡	34.0%	31.3%	0.730
Oophorectomy ‡	0%	2.5%	NA

*** age at registration, † disease duration, ‡ prior to diagnosis of PD, ‡§ coffee/tea cups per day**

52 patients had a family history of PD affecting one or more relatives while 158 patients had no family history of PD and were classified as sporadic PD (Table 4.6). There were no differences between age, disease duration, pre-motor characteristics or any of the risk factors analysed between those with a positive family history of PD compared to those with negative family history of PD (Figure 4.8 and Table 4.6)

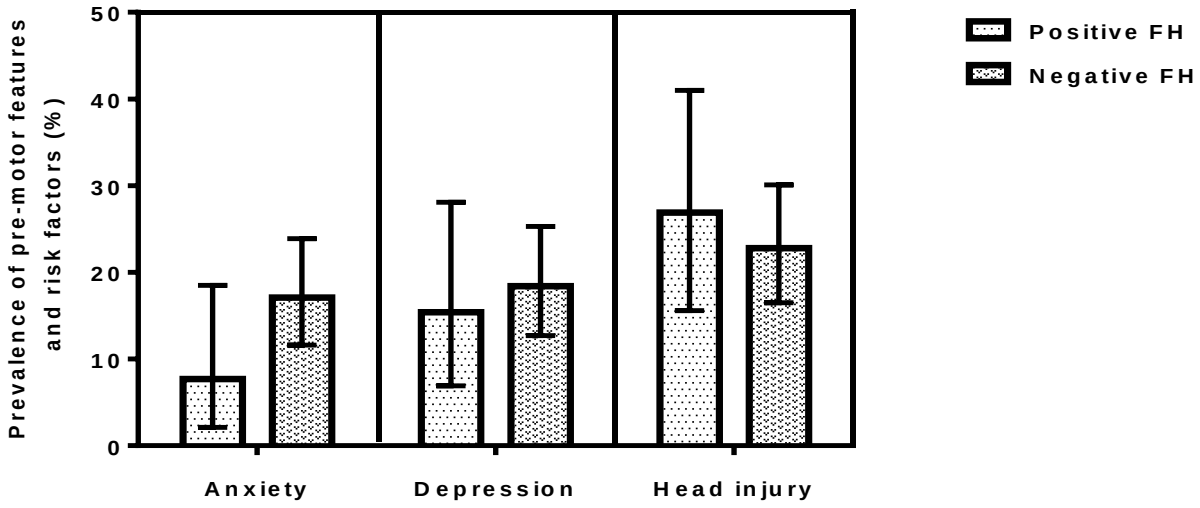

Figure 4.8 shows differences between those with a positive family history of Parkinson's disease (PD), left box of each pair, and those with a negative family history of PD, right box of each pair, in the prevalence of pre-motor features and risk factors in the EOPD cohort of PRoBaND. The top of the boxes represent the proportion of cases who reported anxiety, depression or head injury antedating the diagnosis of PD, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion. None of the differences were significant as shown overlapping confidence intervals in the figure and by p-values >0.05 for the comparisons in Table 4.6.

Table 4.6 Variation in pre-motor characteristics and risk factors in patients (n=210) classified by family history of PD.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	52	158	-
Age* (years)	52.0 (47.4-55.9)	52.7 (47.5-56.6)	0.586
Sex (Males)	63.5%	62.7%	1.000
Duration† (years)	6.0 (3.7-11.3)	7.6 (3.7-11.7)	0.602
Anxiety ‡	7.7%	17.1%	0.117
Depression ‡	15.4%	18.4%	0.681
Head injury ‡	26.9%	22.8%	0.575
Caffeine ‡§	5.5 (3.0-7.8)	5.0 (3-8)	0.368
Smoking ‡	40.4%	29.1%	0.169
Oophorectomy ‡	1.9%	1.9%	1.000

FH = family history, IQR = inter-quartile range, * Age at registration, † disease duration, ‡ prior to diagnosis of PD, ‡§ coffee/tea cups per day

There was no statistically significant difference in the family history, as a risk factor for PD, between those aged less than 40 years at diagnosis PD and those aged greater than 40 years at diagnosis in the EOPD cohort (Figure 4.9 and Table 4.7)

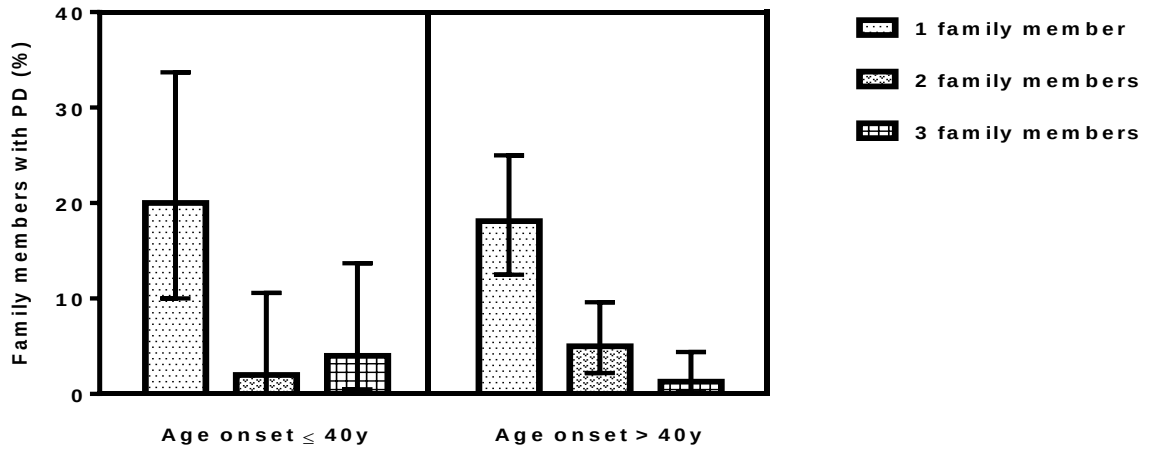

Figure 4.9 shows differences in family history of Parkinson's disease (PD) between those diagnosed at or before the age of 40 years (on the left) and those diagnosed with PD after the age of 40 years (on the right) in the EOPD cohort of PRoBaND. The top of the boxes represent the proportion of cases who reported other family members having been diagnosed with PD, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion. None of the differences were significant as shown by overlapping confidence intervals and p-values > 0.05 in Table 4.7 for the comparisons.

Table 4.7 Family history (as an indication of genetic risk) in EOPD

Family history	**Age of onset ≤ 40y**	**Age of onset > 40y**	**p-value**
Number of cases	50	160	-
Parent(s) affected	12.0%	13.1%	1.000
Sibling(s) affected	0%	1.3%	NA
Parent(s) or sibling(s) affected	12.0%	14.4%	0.816
1 family member affected	20.0%	18.1%	0.835
2 family members affected	2.0%	5.0%	0.689
3 family members affected	4.0%	1.3%	0.241
Any family history of PD	26.0%	24.4%	0.852

PD= Parkinson's disease

4.5 Discussion

The age at onset of PD is variable. In general, individuals with onset of parkinsonian symptoms i.e. tremor, rigidity, bradykinesia, postural instability or combinations thereof, before the age of 20 years are considered to have juvenile onset PD (JOPD) [313], those with onset before the age of 40 or 50 years are classified as having early onset PD (EOPD) or young onset PD (YOPD) [314, 315] and those with onset after the age of 50 years are considered to have late onset PD (LOPD) [316]. A cohort of EOPD patients from the larger PRoBaND study are the subject of analysis of the variation in the clinical expression of PD in this thesis.

There is evidence that the neurodegenerative process that ultimately leads to the death of neurons in PD begins many years, perhaps as long as 5-20 years, before the onset of motor manifestations. Recognizing the early signs of PD, years before the characteristic motor signs appear [317], would be ideal for investigating the role of neuroprotective therapies in such cases, to offset or delay the development of the debilitating effects of PD on mobility and quality of life [318]. Additionally, there can be challenges in diagnosing EOPD when presentation to the general practitioner is with a slight intermittent tremor in a relatively young and otherwise fit person, leading to delays in arriving at the right diagnosis by as many as 15 months compared to LOPD [319]. These considerations make it an interesting proposition to establish whether certain pre-motor features that might act as red flags in someone with an otherwise ordinary looking tremor segregate with some clinical subtypes of PD but not others within the broader rubric of EOPD.

Over the years researchers have recognized several clinical subtypes of PD [320]. Classifying patients into subtypes by predominant motor presentation [179], gender [321] , age of onset [322] and heritability PD [323] can allow for comparison to be made particularly when trying to establish the variation in phenotypic characteristics of the group. Zetusky et al established the idea of clinical subtyping patients with PD because of prognostic implications as they demonstrated in their study of PD patients (n=334), deterioration in mental status was correlated with the PIGD subtype while the TDPD subtype was associated with relative preservation of mental status, earlier age at onset, family history of parkinsonism, and generally a more favourable prognosis [324]. Identification of such subtypes may have

important implications, not only for the reasons mentioned above, but also for generating pathogenic hypotheses and future therapeutic strategies tailored to particular subtypes [325, 326].

In the data set we analysed there were no significant differences in the prevalence of any of the pre-motor features considered here, classifying patients by motor subtype (Table 4.3), gender (Table 4.4) age at onset (Table 4.5) or family history of PD (Table 4.6). This can be interpreted in two ways, first that the whole EOPD cohort that was analysed here was very homogeneous, due to a pre-selection bias, to begin with and therefore the differences in the prevalence of these pre-motor features were not significant or that the differences reported by others [327, 328] are so small that our sample size (n=210) when dichotomised into 2 or divided into 3 groups has small number of cases in each category and the statistical tests used don't have enough power to detect any significant differences.

Furthermore, the only significant differences detected in the risk factors considered here was in the prevalence of smoking between males and females (p=0.046) as well as the reports of oophorectomy in women only in the TDPD motor subtype and those diagnosed with PD after the age of 40 years, when comparing analogous subtypes (Tables 4.3 to 4.5). The higher incidence of smoking in males is a secular trend in the general population in both Scotland and England and could be explained by the higher prevalence of risk taking behaviours in males, while recognising the fact prevalence is also affected by cultural factors [329, 330]. The prevalence recorded in our cohort was generally higher than previously reported figures from NHS patients both for males (37.1% vs. 25.3%) and females (23.1% vs. 20.0%) [331]. The higher figures in our sample could be due to the fact that the population cohort in Simpson et al's paper has a different demographic profile and also due to the fact that our cohort has an inherent pre-selection bias, referred to above, that is an unavoidable fact of most clinic based studies. But the interesting statistic that merits attention is the almost 2:1 ratio of smokers between males and females when considered in the context of smoking being considered a protective influence on the development of PD as reported from epidemiological surveys. If smoking were indeed a protective influence rather than an association, one would expect lesser number of males in this cohort to be affected by PD; however, our results replicate the well reported finding of more males to be affected by PD than females (Table 4.4). Bilateral oophorectomies are usually performed as part of a procedure, hsyterosalpingo-oophorectomy, and this radical procedure is performed more in

older women than younger women as prevalence of hormone driven malignancies increases with age [332]. This could explain the findings from our study. However, it would not explain why only women in the TDPD cohort reported oophorectomies, but it would be unreasonable to jump to conclusions or make educated guesses given the total number of women who reported oophorectomies in the whole cohort is small. It would also be unreasonable to dismiss it altogether before analysing this finding in a larger study.

One can therefore surmise that environmental risk factors of PD considered here, except oophorectomy, don't contribute significantly to the classification of patients by clinical subtypes. Therefore, as a corollary, influence of genetic factors, the underlying biologic makeup, iatrogenic influences or the distribution of alpha-synuclein in the brain may have a greater role to play than environmental factors in the divergence of trajectories in the prognosis, as used in its broadest meaning, of the clinical subtypes of PD.

The strongest associations with later diagnosis of PD amongst all risk factors, genetic or environmental, in a recent meta-analysis were a family history of a first-degree or any relative with PD (odds ratio [OR], 3.23; 95% confidence interval [CI], 2.65–3.93 and OR, 4.45; 95% CI, 3.39–5.83) [194]. A positive family history of PD, particularly when traced across more than one generation, would suggest a hereditary trait passed on from parent to offspring in their genes. A hereditary trait therefore has connotations of a genetic aetiology. Our results did not show any significant difference in the family histories between those with age of onset of PD before 40 years of age and those who had onset of PD symptoms after the age of 40 years in the EOPD cohort (Table 4.7), to suggest that more cases in one group or the other had hereditary parkinsonism. One could therefore, on the basis of these results, argue that the arbitrary cut-off of 40 years, as opposed to 50 years, in defining EOPD, with the tacit assumption that cases with PD diagnosed before the age 40 years as opposed to 50 years are likely to have a genetic basis to PD, is an artificial construct. The counter argument could be that the numbers in our study are relatively small to draw such a conclusion and that autosomal recessive genes like *Parkin* usually have an earlier age of onset and no family history of PD in the previous generation.

4.6 Conclusion

There were no statistically significant differences in the pre-motor symptoms such as anxiety or depression and environmental risk factors such as head injury and caffeine consumption between the clinical subtypes in the EOPD cohort. Smoking was more prevalent in males compared to females whilst oophorectomy was only reported by women in the TDPD motor subtype and those with aged 40 years or older at onset of PD, when comparing to analogous subtypes. Family histories suggesting a hereditary nature of PD did not differ between those diagnosed before or after the age of 40 years in our EOPD cohort.

Chapter 5. Variation in motor symptoms of EOPD

5.1 Objective

The objective in this chapter is to analyse the variation in the motor symptoms in a cohort of patients with early onset Parkinson's disease from the PRoBaND study.

5.2 Introduction

The diagnostic criteria for Parkinson's disease (PD) have always been biased in favour of its motor manifestations, not only because these were the first to be recognized as a distinct symptom complex by James Parkinson [7], but also due to the fact that these protean manifestations contribute significantly to the disability caused by the condition. There are several motor subtypes of PD. Tremor dominant PD (TDPD) and postural instability gait difficulty (PIGD) represent two ends of the spectrum and some patients have features that have semblance to both motor subtypes yet don't meet the formal criteria for either, hence classified as 'Mixed' or 'Indeterminate', terms used interchangeably. There are only hypothesised biological differences between these motor subtypes but we know from clinical observations they certainly have different disease trajectories and responses to treatment, hence it sounds logical to use this framework as one of the ways of classifying PD patients.

5.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0). Data required for MDS-UPDRS scores and Hoehn Yahr (HY) staging were recorded at study visit 1. Historical presenting features were recorded from case notes in the case report form (CRF)

at study visit 2 as per study protocol. Problems affecting balance and speech subjectively and objectively were recorded from MDS-UPDRS part 2 and MDS-UPDRS part 3 respectively.

The motor scores recorded by the clinician on MDS-UPDRS part 3 were in a practically defined 'on' state. It was not considered convenient for patients enrolled in the study to withhold their anti-parkinson medications in the morning to induce an 'off' state as their clinic visits could have been scheduled anytime between 9:00 am and 4:30 pm and this would risk putting patients through a difficult phase for hours together if their 'off' state was disabling. A practically defined 'on' state for the purpose of this non-interventional study was defined as patients taking their normally prescribed anti-parkinson medications, including rescue medications, at their pre-scheduled times to have sufficient dopamine leves in their system to function as closely possible as they would in their normal daily lives.

To analyse the variation in the motor phenotype of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed'), gender, age at onset of PD symptoms and heritability of the Parkinsonian trait as described in Chapter 4.

Levodopa equivalent daily dose (LEDD) dose was calculated using the following dose equivalence: Levodopa 100 mg = Controlled Release Levodopa 70 mg = Ropinirole 5 mg = Rotigotine 5mg = Pramipexole 1mg (salt) = Bromocriptine 10mg = Pergolide 1mg = Cabergoline 1 mg = Selegiline 10mg = Zelapar 1.25mg = Rasagiline 1 mg as baseline = 10mg Apomorphine (visit 0). All medication equivalent doses, levodopa equivalent units (LEU) as mg/day, were added together to obtain LEDD. Where a COMT inhibitor was used, this would add 30% to the levodopa dose used to calculate LEDD [333, 334] .

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the

Kruskal Wallis test statistic showed a significant difference between the 3 groups, correcting for multiple comparisons in the post hoc tests with Dunn's correction. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data, with post-hoc tests as appropriate.

Generalised linear modelling, based on ANCOVA, was used with age, disease duration and LEDD as covariates to determine the effect of the motor subtype, gender, age at onset and hereditary versus sporadic parkinsonism (independent variables) on the UPDRS part 3 scores (dependent variable). Age and disease duration were used as covariates in the statistical models to determine the effect of the motor subtype, gender, age at onset and hereditary versus sporadic parkinsonism (independent variables) on the HY stage (dependent variable). Logarithmic transformations (base 10) of numerical data were used where tests of normality (Shapiro Wilk) failed.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (version *23 for Windows*; IL, USA).

5.4 Results

276 patients with EOPD (age of onset <50 years) from the PRoBaND study who had completed all questionnaires relevant to the data analysis in this chapter were included (Figure 5.1).

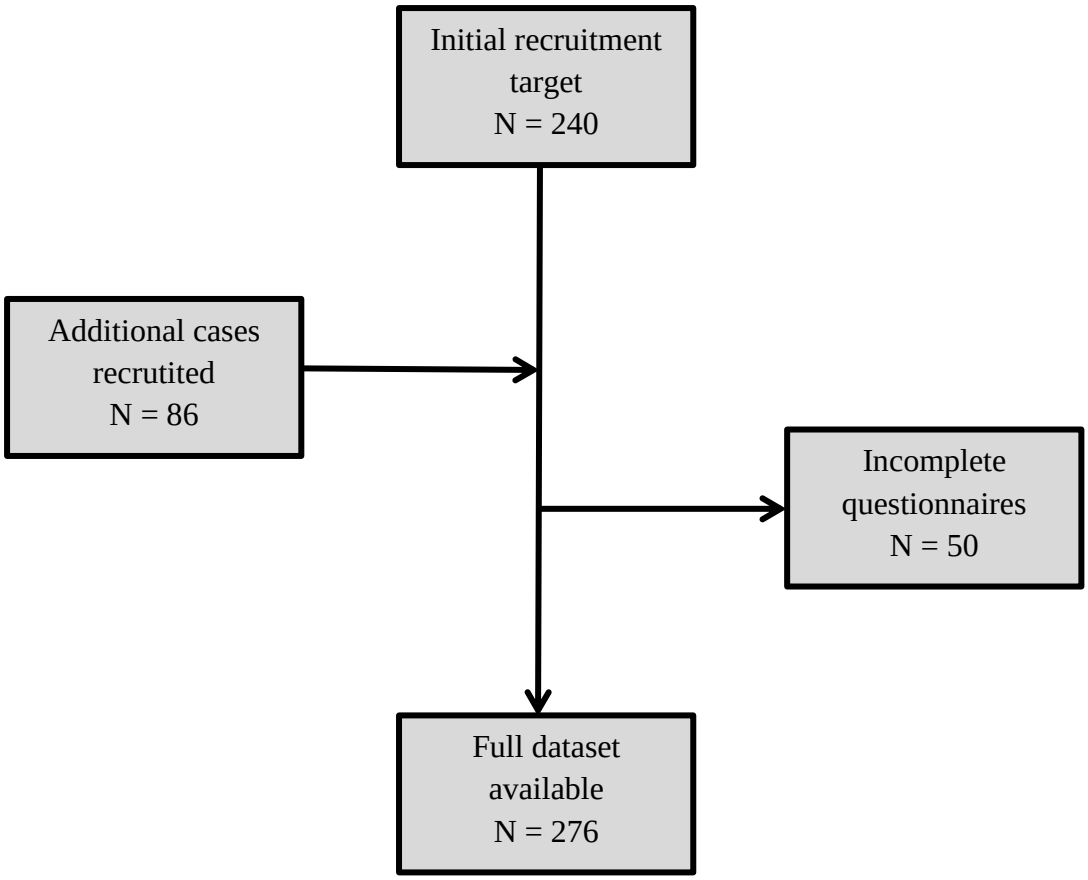

Figure 5.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The median age at the time of registration was 51.4 years (inter-quartile range, IQR, 46.9-56.3) and median disease duration was 6.8 years (IQR 2.7-11.5). Two- thirds of the patients were males and the vast majority were Caucasians (Figure 5.2). Other demographic variables of these 276 patients are presented in Table 5.1

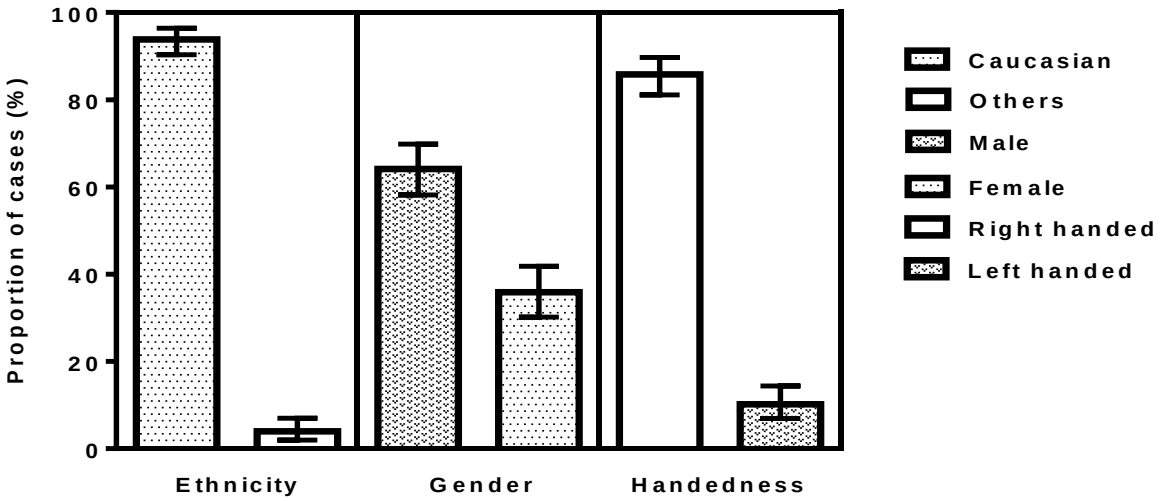

Figure 5.2 shows the demographic profile of the early onset PD cohort (n=276) used in this analysis with proportion of cases on the y-axis and demographic characteristics on the x-axis.

Table 5.1 Demographic profile of the early onset PD patients (n=276) analysed in this section who had completed all relevant questionnaires.

Variable	**Median (IQR)**
Number of cases	276
Age (at registration, in years)	51.4 (46.9-56.3)
Sex (males)	67.7%
Height (metres)	1.7 (1.7-1.8)
Weight (kg)	75.0 (58.0-90.0)
Handedness	85.8%
Married	66.9%
Employed (at the time of diagnosis)	89.9%
Ethnicity	
Caucasian	93.8%
Asian	3.6%
African	0%
Caribbean	0.4%
Others (including Romas)	0.4%
Disease duration (years)	6.8 (2.7-11.5)

IQR = inter-quartile range.

The variation in the presenting features, motor symptoms, medication requirements, UPDRS motor scores and Hoehn Yahr staging in patients classified by motor subtype is shown below (Figures 5.3 and 5.4, Table 5.2).

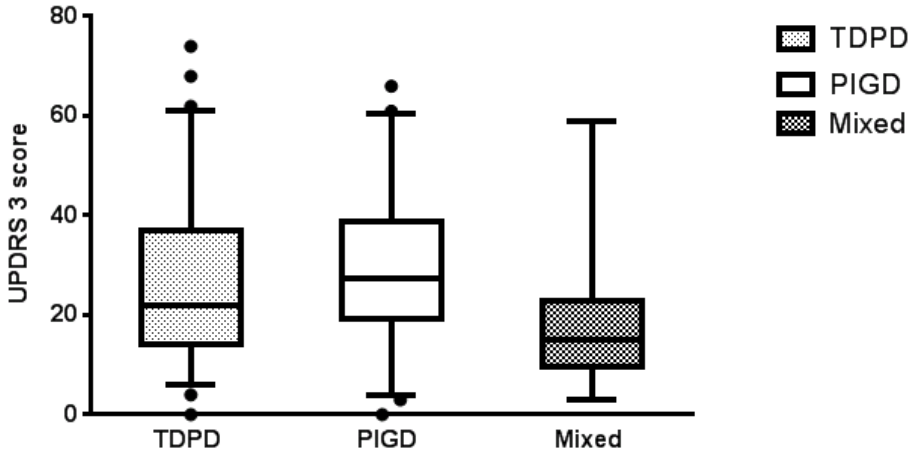

Figure 5.3 shows the unified Parkinson's disease rating scale part 3 (UPDRS 3) scores (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. UPDRS 3 scores were significantly higher in those with PIGD (n=100) compared to those with 'Mixed' (n=21) motor subtype (p=0.014) when corrected for age, disease duration and medication usage but not to TDPD (n=155).

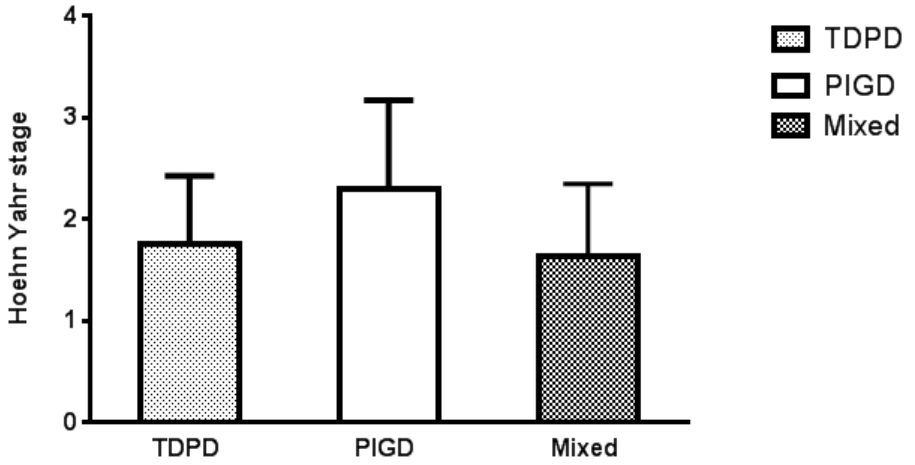

Figure 5.4 shows the Hoehn Yahr (HY) stage (on the y-axis) and the motor subtype (on the x-axis) of the patients. The top of the boxes represent the medians and the whiskers the inter-quartile ranges (bottom whisker is hidden in the box). The HY stage was significantly lower in those with TDPD (n=155) compared to those with the PIGD (n=100) motor subtype when corrected for age and disease duration ($p<0.001$).

Table 5.2 Variation in motor symptoms, medication requirements, UPDRS 3 score and Hoehn Yahr staging in patients (n=276) classified by motor subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number of cases	155	100	21	-
Age* (years)	50.4 (45.1-54.3)	54.6 (48.8-59.8)	49.6 (46.9-52.5)	**<0.001**
Sex (males)	64.1%	59%	65%	0.356
Duration (years)	5.1 (2.0-9.6)	9.1 (4.6-15.0)	7.4 (2.5-11.8)	<0.001
Side of onset (r)	48.5%	56.3%	38.9%	0.355
Presenting features (based on clinical notes at the time of diagnosis of PD)				
Resting tremor	80.3%	59.3%	77.8%	**0.003**
Bradykinesia	84.1%	78.5%	83.3%	0.583
Rigidity	78.9%	85.2%	83.3%	0.511
Post. instability	14.4%	28.6%	11.1%	**0.028**
Balance problems†	77.4%	95.0%	85.7%	**<0.001**
Balance problems‡	21.3%	56.0%	23.8%	**<0.001**
Speech problems†	65.8%	83.0%	57.1%	**0.004**
Speech problems‡	56.8%	75.0%	42.9%	**0.002**
LEDD (mg/day)	599 (300-965)	840 (532-1043)	738 (538-1053)	**0.003**
UPDRS 3 scores	22 (14-36)	27 (19-38)	15 (10-21)	**0.014**
Hoehn Yahr stage	2.0 (1.0-2.0)	2.0 (2.0-3.0)	1.5 (1.0-2.0)	**<0.001**

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, IQR = inter-quartile range, * Age at registration, (r) = right side, † = subjective from MDS-UPDRS Part 2, ‡ = objective from MDS-UPDRS Part 3, LEDD = levodopa equivalent daily dose, UPDRS 3 = MDS-UPDRS scale Part 3

There were significant differences in the presenting features of the 3 motor subtypes with resting tremor, as expected and by definition, being most common in the TDPD group compared to other motor subtypes (p=0.003) and postural instability least prevalent at presentation in the 'Mixed' motor subtype (p=0.024) (Table 5.1) although it is important to point out that the 4 cardinal features required to diagnose PD i.e. resting tremor, bradykinesia, rigidity and postural instability were present at diagnosis in all groups, albeit in varying proportions. Post hoc tests, showed the differences in the prevalence of resting tremor at presentation to be present between TDPD and PIGD motor subtypes (p=0.001) with no statistically significant differences between TDPD and 'Mixed' motor subtypes (p=0.759) or between PIGD and 'Mixed' motor subtypes (p=0.183). Post hoc tests for postural instability, deemed by the clinician to be present at diagnosis, showed significant differences between TDPD and PIGD (p=0.018) with no statistically significant differences between TDPD and 'Mixed' motor subtypes (p=0.707) or between PIGD and 'Mixed' motor subtypes (p=0.146).

There were also significant differences in the prevalence of balance problems at the time of assessment in the PRoBaND study both subjectively, based on UPDRS Part 2 (p<0.001) and objectively, based on UPDRS Part3 (p<0.001) in between the three motor subtypes, these being most prevalent in the PIGD group (Table 5.1). Post hoc tests of subjective balance problem showed the differences to be present only between TDPD and PIGD (p<0.001) but not between TDPD and 'Mixed' (p=0.573) or PIGD and 'Mixed' (p=0.141). Post hoc tests of objective balance problems showed the differences between TDPD and PIGD (p=<0.001) and PIGD and 'Mixed' (p=0.009) but not between TDPD and 'Mixed' (p=0.781).

Further, there were significant differences in the prevalence of speech problems both subjectively (p=0.004) and objectively (p=0.002) in between the groups, these being most prevalent in the PIGD group (Table 5.1). Post hoc tests showed that speech problems subjectively were more prevalent in the PIGD group compared to the TDPD group (p=0.003) and PIGD compared to the 'Mixed' group (p=0.017) but there were no significant differences between the TDPD group and the 'Mixed' group (p=0.470) (Figure 5.5). Post hoc tests showed that speech problems objectively were more prevalent in the PIGD group compared to the TDPD group (p=0.003) and PIGD compared to the 'Mixed' group (p=0.007) but there

were no significant differences between the TDPD group and the 'Mixed' group (p=0.250) (Figure 5.5).

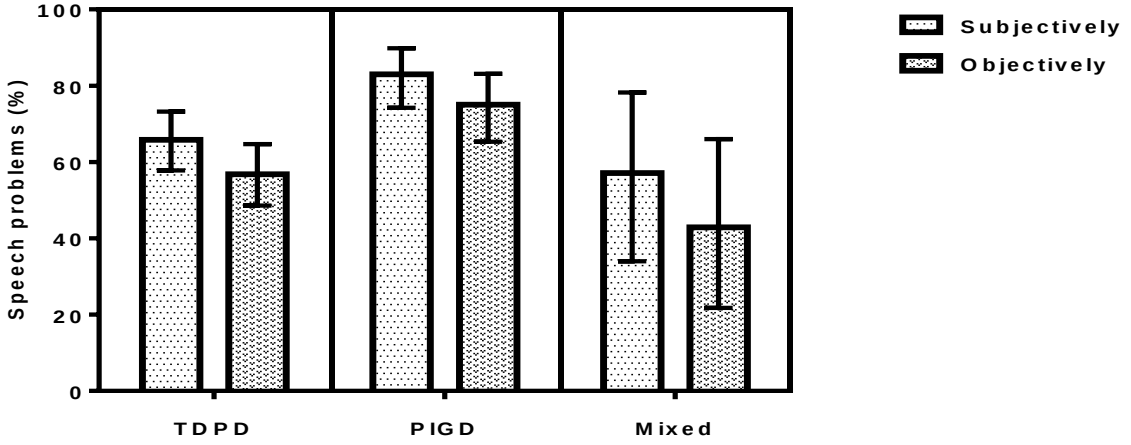

Figure 5.5 shows the proportion of patients who had speech problems (subjectively) on the Unified Parkinson's disease rating scale Part 2 (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with speech problems, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

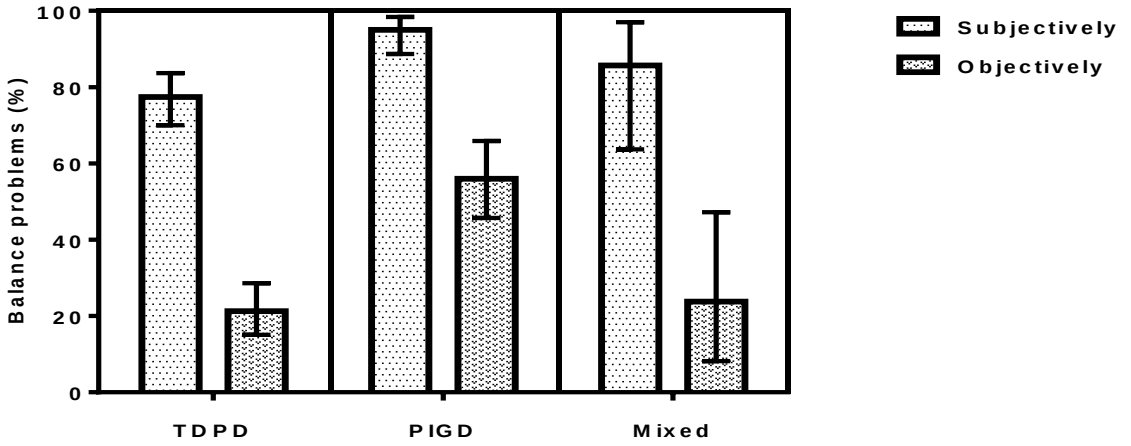

Figure 5.6 shows the proportion of patients who had balance problems (objectively) on the Unified Parkinson's disease rating scale Part 3 (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with balance problems, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

There were also significant differences between 3 groups in UPDRS 3 scores (p=0.014) and Hoehn Yahr stages (p<0.001) after correcting for differences in age, disease duration and LEDD in statistical models (Table 5.2).

Post hoc tests, with Dunn's correction for multiple comparisons, showed the differences in UPDRS 3 to be present between PIGD and 'Mixed' motor subtypes with significantly lower UPDRS 3 scores in the 'Mixed' motor subtype while the HY stages showed significant differences to be between TDPD and PIGD groups.

Speech problems were more prevalent in males compared to females both subjectively based on UPDRS 2 and objectively based on UPDRS 3 scoring but there were no statistically significant differences between males and females on any of the motor signs at presentation or problems that developed later on (Table 5.3) including their UPDRS3 scores (Figure 5.7), HY stages (Figure 5.8) or the proportion of males and females classified by motor type (5.9)

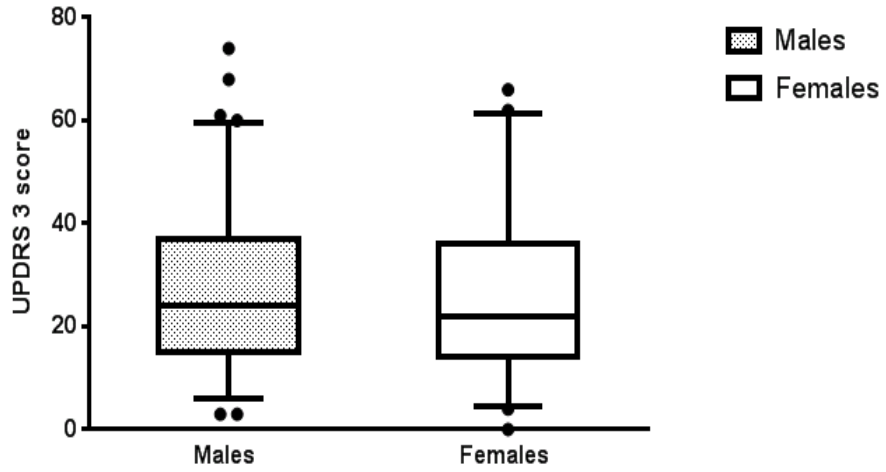

Figure 5.7 shows the unified Parkinson's disease rating scale part 3 scores (on the y-axis) and the gender (on the x-axis). The line in the centres of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between males (n=177) and females (n=99) (p=0.549)

Table 5.3 Variation in motor symptoms, medication requirements, UPDRS 3 score and Hoehn Yahr staging in patients (n=276) classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p-value**
Number of cases	177	99	-
Age(at registration, in years)	51.7 (46.5-56.8)	51.1 (47.2-55.6)	0.968
PD duration (years)	7.3 (2.8-11.5)	6.5 (2.6-11.9)	0.737
Side of onset (right)	50.7%	50.0%	0.922
Presenting features (based on clinical notes at the time of diagnosis of PD)			
Resting tremor	72.7%	73.2%	0.941
Bradykinesia	79.2%	87.5%	0.118
Rigidity	82.8%	79.0%	0.481
Postural instability	21.2%	14.5%	0.222
Motor subtype			
TDPD	59.3%	50.5%	0.156
PIGD	33.3%	41.4%	0.180
'Mixed'	7.3%	8.1%	0.824
Balance problems (subjective)	85.9%	81.8%	0.372
Balance problems (objective)	29.9%	41.4%	0.053
Speech problems (subjective)	78.0%	59.6%	**0.001**
Speech problems (objective)	67.8%	52.5%	**0.012**
LEDD (mg/day)	760 (400-1030)	600 (328-986)	0.136
UPDRS 3 scores	24 (15-37)	22 (15-35)	0.549
Hoehn Yahr stage	2.0 (1.5-2.5)	2.0 (1.3-2.5)	0.639

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, IQR = inter-quartile range, * Age at registration, (r) = right side, † = subjective from MDS-UPDRS Part 2, ‡ = objective from MDS-UPDRS Part 3, LEDD = levodopa equivalent daily dose, UPDRS 3 = MDS-UPDRS scale Part 3

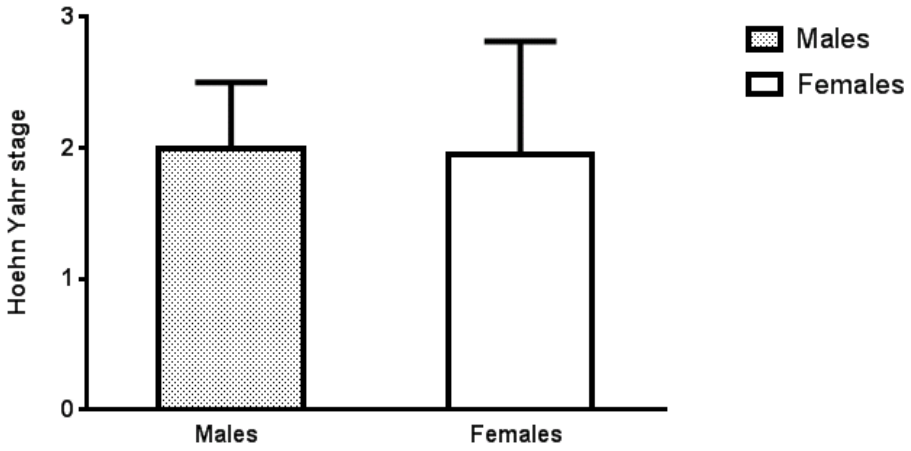

Figure 5.8 shows the Hoehn Yahr stage (on the y-axis) and gender (on the x-axis) of the patients. The top of the boxes represent the medians and the whiskers the inter-quartile ranges. No significant differences were found between males (n=177) and females (n=99) (p=0.871)

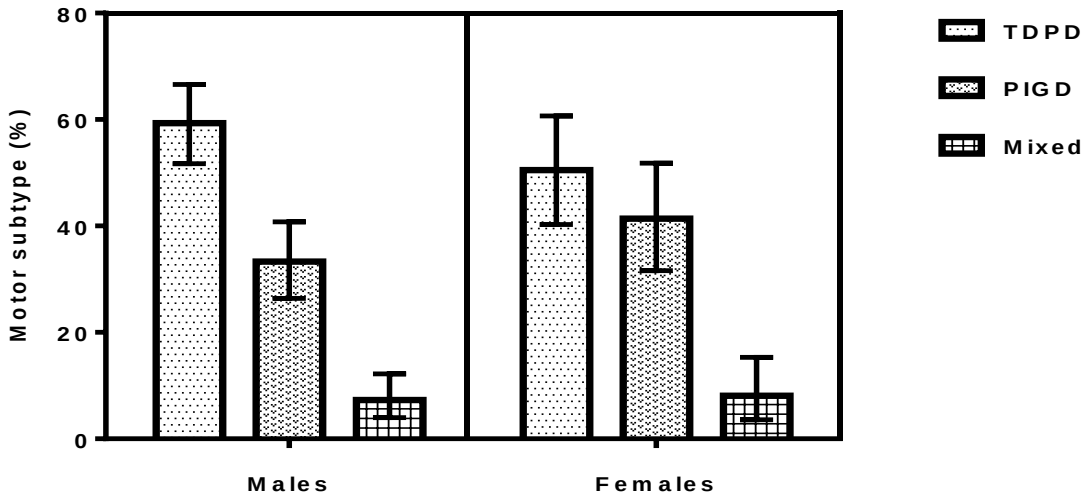

Figure 5.9 shows the motor subtype (on the y-axis) and their gender (on the x-axis) of the patients (n=276) in the EOPD cohort of the PRoBaND study. The top of the boxes represent the proportion of cases of each motor subtype, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion. There were no significant differences in the proportion of those classified as TDPD (p=0.156), PIGD (p=0.180) or 'Mixed' (p=0.824) between the 2 genders.

There was no difference in the proportion of cases classified as TDPD (p=0.170), PIGD (p=0.712) or 'Mixed' (p=0.063) between those diagnosed with PD aged less than (or equal to) 40 years compared to those diagnosed after the age of 40 years in the EOPD cohort after correcting for age in the statistical model (Figure 5.10). There were no other statistically significant differences in between the 2 subgroups in the UPDRS3 scores (Figure 5.11), Hoehn Yahr stages (Figure 5.12) or the prevalence of motor manifestations either at presentation or those that developed later on (Table 5.4).

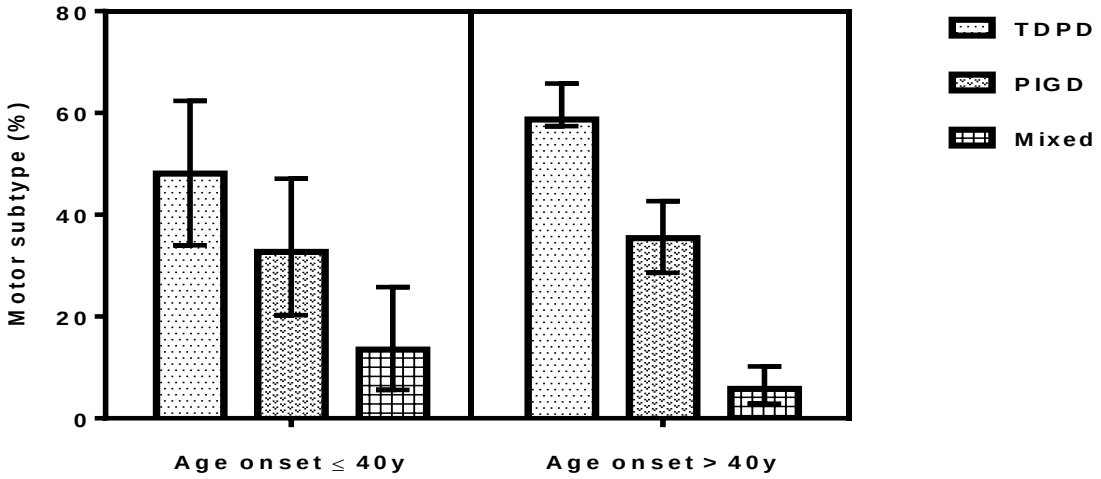

Figure 5.10 shows the proportion of patients with the tremor dominant Parkinson's disease (TDPD), postural instability gait difficulty (PIGD) and "Mixed" motor subtypes on the y-axis and age at onset of PD symptoms on the x-axis of the patients (n=276) in the EOPD cohort of the PRoBaND study. The top of the boxes represent the proportion of cases of each motor subtype, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion. There were no significant differences in the proportion of cases classified as TDPD (p=0.170), PIGD (p=0.712) or 'Mixed' (p=0.063) between those age at diagnosis ≤ 40 years and those with age at diagnosis > 40 years.

Table 5.4 Variation in motor symptoms, medication requirements, UPDRS 3 score and Hoehn Yahr staging in patients (n=276) classified age at onset of PD.

Variable	**Age onset ≤ 40 years** median (IQR)	**Age onset > 40 years** median (IQR)	**p-value**
Number of cases	64	212	-
Age (at registration, in years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	**<0.001**
Sex (Males)	67.2%	63.2%	0.561
PD duration (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.707
Side of onset (right)	52.0%	50.3%	0.802
Presenting features (based on clinical notes at the time of diagnosis of PD)			
Resting tremor	73.1%	72.0%	0.971
Bradykinesia	85.1%	81.1%	0.546
Rigidity	82.4%	81.4%	0.854
Postural instability	19.2%	18.6%	0.952
Motor subtype			
TDPD	48.1%	58.7%	0.170
PIGD	32.7%	35.5%	0.712
Mixed	13.5%	5.8%	0.063
Balance problems (s)	90.6%	81.8%	0.118
Balance problems (o)	35.9%	33.2%	0.717
Speech problems (s)	79.7%	68.7%	0.093
Speech problems (o)	71.9%	59.3%	0.071
LEDD (mg/day)	700 (363-998)	715 (400-1008)	0.659
UPDRS 3 scores	25 (18-40)	23 (14-34)	0.725
Hoehn Yahr stage	2.0 (1.0-2.5)	2.0 (1.5-2.5)	0.871

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, IQR = inter-quartile range, * Age at registration, (r) = right side, † = subjective from MDS-UPDRS Part 2, ‡ = objective from MDS-UPDRS Part 3, LEDD = levodopa equivalent daily dose, UPDRS 3 = MDS-UPDRS scale Part 3

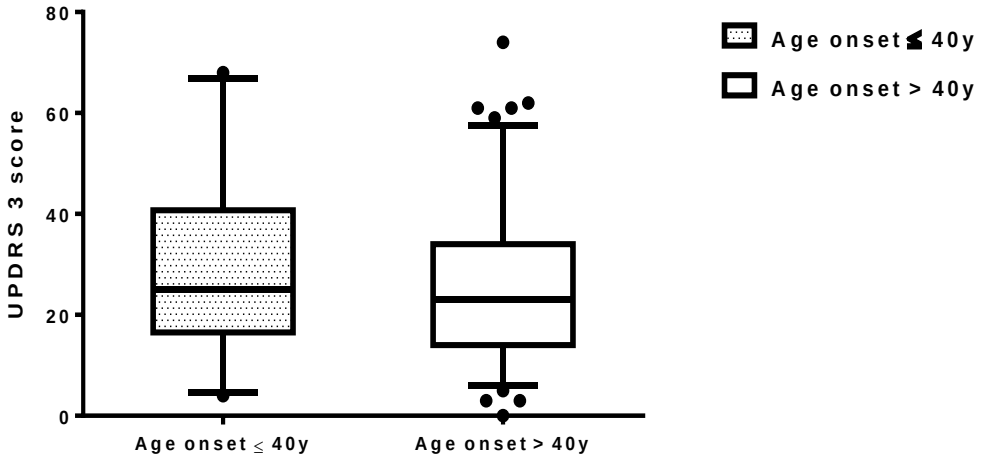

Figure 5.11 shows the unified Parkinson's disease rating scale part 3 scores (on the y-axis) and the age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with age at onset of PD ≤ 40 years (n=64) compared to those with age at onset of PD > 40 years (n=212) (p=0.725)

Figure 5.12 shows the Hoehn Yahr stage (on the y-axis) and the age at onset of PD (on the x-axis). The top of the boxes represent the medians and the whiskers the inter-quartile ranges. No significant differences were found between those with age at onset of PD ≤ 40 years (n=64) compared to those with age at onset of PD > 40 years (n=212) (p = 0.871)

The only differences in the motor presentation of those with a positive family history of PD compared to cases with no family history of PD were that resting tremor at presentation, as one of the 4 cardinal signs of PD when using Queen Square Brain Bank criteria for diagnosis, was more commonly found in those with hereditary PD (p=0.005) while bradykinesia at presentation was more commonly present in those with sporadic PD (p=0.030) (Table 5.5). There were no other statistically significant differences in between the 2 subgroups in the UPDRS3 scores (Figure 5.13), Hoehn Yahr stages (Figure 5.14), motor subtypes (Figure 5.15) or the prevalence of motor manifestations either at presentation or those that developed later on (Table 5.5).

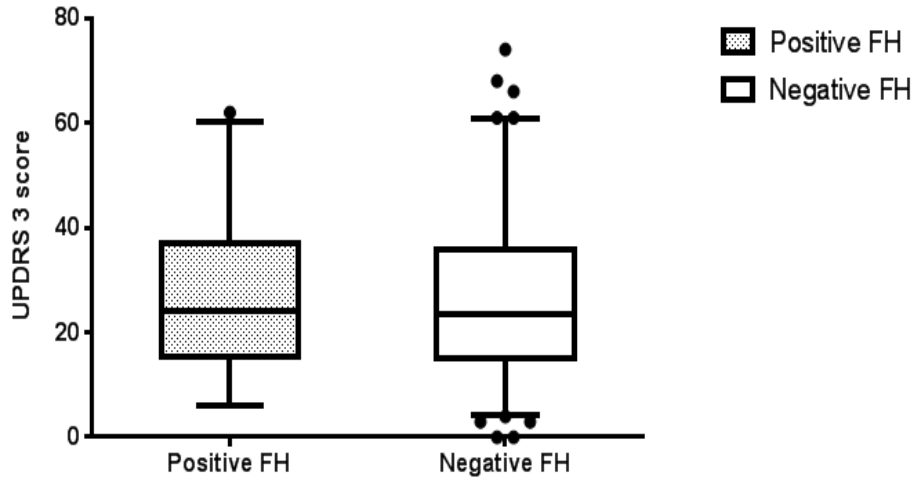

Figure 5.13 shows the Unified Parkinson's disease rating scale Part 3 score of the cases (on the y-axis) and their family history (FH) of Parkinson's disease (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with a positive family history of PD (n=64) compared to those with no family history of PD (n=212) (p=0.987)

Table 5.5 Variation in motor symptoms, medication requirements, UPDRS 3 score and Hoehn Yahr staging in patients (n=276) classified by family history.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	64	212	-
Age(at registration, in years)	52.2 (47.3-56.8)	51.2 (46.9-56.1)	0.582
Sex (Males)	65.6%	63.7%	0.776
PD duration (years)	6.7 (3.5-12.1)	6.8 (2.5-11.3)	0.312
Side onset (right)	55.4.%	49.2%	0.485
Presenting features (based on clinical notes at the time of diagnosis of PD)			
Resting tremor	84.5%	68.3%	**0.005**
Bradykinesia	73.2%	85.1%	**0.030**
Rigidity	80.4%	82.0%	0.692
Postural instability	18.5%	18.9%	0.952
Motor subtype			
TDPD	56.3%	56.1%	0.987
PIGD	34.4%	36.8%	0.724
Mixed	9.4%	7.1%	0.543
Balance problems (subjective)	80.3%	84.9%	0.686
Balance problems (objective)	36.4%	33.0%	0.507
Speech problems (subjective)	66.7%	72.6%	0.398
Speech problems (objective)	69.7%	59.9%	0.132
LEDD (mg/day)	824 (460-1033)	655 (400-1000)	0.135
UPDRS 3 scores	23 (15-36)	23 (15-35)	0.987
Hoehn Yahr stage	2.0 (1.5-2.0)	2.0 (1.5-2.5)	0.333

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, IQR = inter-quartile range, * Age at registration, (r) = right side, † = subjective from MDS-UPDRS Part 2, ‡ = objective from MDS-UPDRS Part 3, LEDD = levodopa equivalent daily dose, UPDRS 3 = MDS-UPDRS scale Part 3

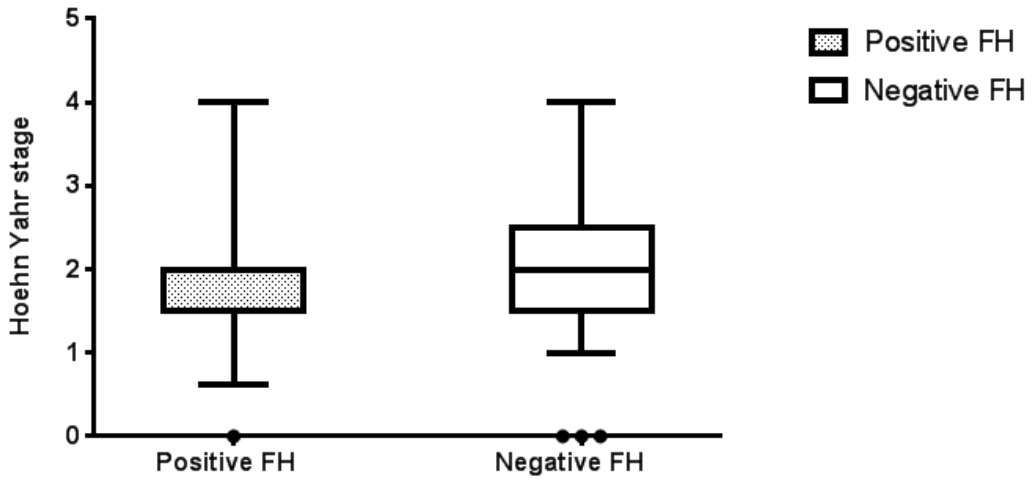

Figure 5.14 shows the Hoehn Yahr stage (on the y-axis) and the family history of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with familial PD (n=64) compared to those with sporadic PD (n=212) (p=0.333)

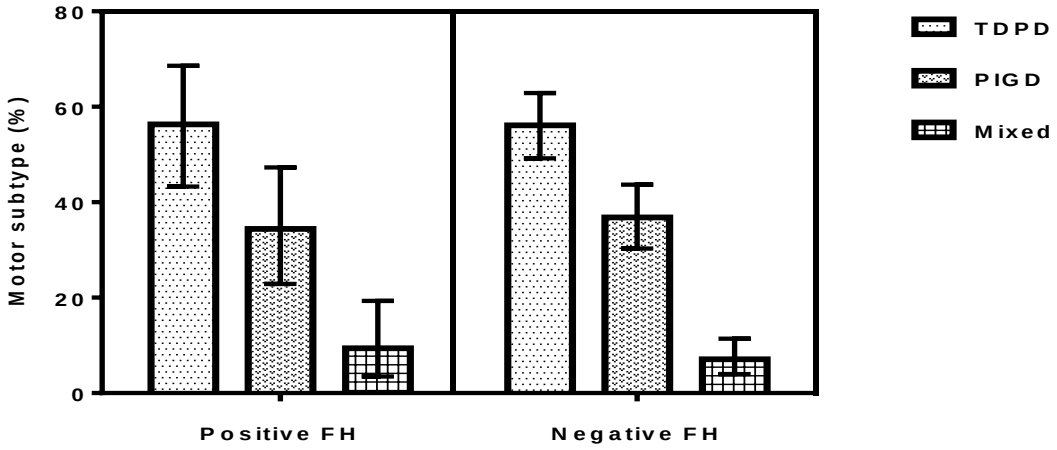

Figure 5.15 shows the proportion of patients with the tremor dominant Parkinson's disease (TDPD), postural instability gait difficulty (PIGD) and "Mixed" motor subtypes on the y-axis and family history (FH) of PD on the x-axis of the patients (n=276) in the EOPD cohort of the PRoBaND study. The top of the boxes represent the proportion of cases of each motor subtype, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

5.5 Discussion

Patients with PD show marked heterogeneity in their motor features not only in relation to the motor subtype but also some differences when classifying patients by gender, age at onset of PD and hereditary versus sporadic PD.

This variation in the motor presentation could have several possible explanations. Some have hypothesized that TDPD and PIGD motor subtypes of PD might be due to differential iron load within different basal ganglia structures in the brain [335]. Others have suggested that abnormal oscillatory activity in different neuronal circuits, some localised to the basal ganglia, might explain the differences in the motor presentations [336].

Pathological specimens from post-mortem studies have demonstrated that the motor subtypes may have different morphological lesion patterns. PIGD cases showed more severe cell loss in the ventrolateral part of substantia nigra pars compacta that projects to the dorsal putamen than the medial part projecting to caudate nucleus and anterior putamen. Reduced dopaminergic input causes overactivity of the GABA ergic inhibitory striatal neurons projecting via the "indirect loop" to substantia nigra zona reticulata and medial pallidum leading to inhibition of the glutamatergic thalamo-cortical motor loop and reduced cortical activation. TDPD cases showed more severe neuron loss in medial than in lateral substantia nigra zona compacta and damage to the retrorubral field A8, containing only few tyrosine hydroxylase and dopamine transporter immunoreactive neurons. The retrorubral field A8 that is rather preserved in PIGD [337] .

Gender differences have been reported in motor symptoms [327] with resting tremor at presentation more common in women. We didn’t find this difference in the EOPD cohort but we found speech problems less prevalent in women compared to men both subjectively ($p=0.001$) and objectively ($p=0.012$) as recorded on the UPDRS part 2 and 3 scores. The gender differences may be related to the effects of female sex hormones such as estradiol on synaptic plasticity, neurotransmission, neurodegeneration, and cognitive circuits in the brain leading to notable sex differences in the incidence and manifestations of several central nervous system disorders, including Parkinson's disease [338].

The age at onset can influence the motor phenotype of PD. Tremor at presentation is reported to be twice as common in those with older onset as compared to those with younger onset, the prevalence increasing linearly with age at onset of PD [339]. Those with younger age at onset also have a longer disease course and slower progression [314]. Our results did not show that tremor (p=0.971), bradykinesia (p=0.546), rigidity (p=0.854) or postural instability (p=0.952) as presenting features were more common in those diagnosed with PD before the age of 40 years compared to those diagnosed after the age of 40 years in the EOPD cohort. There was also no significant difference in the Hoehn Yahr stage between those diagnosed with PD before the age of 40 years compared to those diagnosed after the age of 40 years (p=0.871) when corrected for age and disease duration, suggesting the rate of motor progression was similar in the two groups. This is not in concurrence with Schrag et al's [314] observations probably due to the fact that the populations analysed in the two studies are different. Their cohort had 10 with juvenile parkinsonism (onset before age 21 years) and the rest of 139 cases had age of onset between 21 to 40 years. Those with juvenile parkinsonism very likely either had *Parkin* mutations or had secondary parkinsonism due to some other aetiology such as SCA mutations as idiopathic Parkinson's disease is quite rare in that age group in clinical practice. None was genetically tested to rule out other causes of parkinsonism except one who had a SCA3 mutation.

A smaller prospective study comparing familial PD (n=50) with sporadic PD (n=50), after excluding those cases with genetic mutations causing PD (n=9) reported similar motor signs and symptoms between the 2 groups suggesting that either the topographic distribution of the neurodegenerative process in the brain leading to the parkinsonian clinical syndrome could be similar in these 2 groups or the aetiologies could be similar [340]. A larger study of familial PD (n=40) and sporadic PD (n=1277) also reported similarities in the phenotypic characteristics, such as tremor as the initial motor symptom and asymmetric parkinsonism during disease course, of the two groups with similar conclusions [341]. Our results also showed no major differences in between the 2 subgroups, as previously reported, but in our cohort tremor at presentation was more common in those with familial PD (p=0.005) while bradykinesia at presentation was more common at presentation in those with sporadic PD (p=0.030). The definition of familial PD in the larger study (with 10 or more affected members) was different from ours where any family history of PD in first relations across 3 generations was considered as inherited trait.

5.6 Conclusion

The differences in the 3 motor subtypes, as one would expect, showed differences in the prevalence of tremor and postural instability but there were also other significant differences of note between TDPD and PIGD motor subtypes. Speech problems both subjectively and objectively were most prevalent in the PIGD subcases. This would not be surprising if speech is considered a midline or axial problem that would go hand in hand with other axial problems such as problems with balance. UPDRS 3 scores after correcting for age, disease duration and LEDD were the lowest in the TDPD cases. There were also some notable differences when cases were classified by gender, with males experiencing more speech problems both subjectively and objectively despite no significant differences in age or disease duration. More patients who had age at onset of PD less than 40 years had the PIGD motor subtype whereas more patients who had onset of PD after age of 40 years in the EOPD cohort had the TDPD phenotype.

Chapter 6. Variation in motor complications in EOPD

6.1 Objective

The objective in this chapter is to analyse the variation in the prevalence of motor complications in a cohort of patients with early onset Parkinson's disease.

6.2 Introduction

Motor complications in the form of levodopa induced dyskinesia, 'off' period dystonia, 'on' 'off' fluctuations and freezing of gait are often seen either as a complication of long-term dopaminergic therapy or difficult to treat problems that do not respond well to changes in medications, nevertheless, cause problems that significantly affect the quality of life of these patients. These complications typically develop after 4-6 years of therapy, and affect approximately half of all patients but there is a recognised heterogeneity in the prevalence and severity of these complications in the population of patients with PD.

6.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data and anti-parkinsonian medication requirements in levodopa equivalent untis (LEU) in mg/day, at baseline (visit 0). Levodopa equivalent daily dose (LEDD) was calculated using the formula described in Chapter 5. Data required for MDS-UPDRS Part 4 (UPDRS4) scores for evaluation of complications of dopaminergic therapy including the presence of dyskinesia, 'on' 'off' fluctuations, 'off' dystonia were recorded at study visit 1. Freezing was recorded from clinician completed UPDRS3 forms.

To analyse the variation in motor complications of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed') , gender , age at onset of PD symptoms and heritability of the Parkinsonian trait as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data, with post-hoc tests as appropriate.

In generalised linear models, based on ANCOVA, age, disease duration and LEDD were used as covariates to determine the effect of the motor subtype, gender, age at onset and family history of PD (independent variables) on the UPDRS4 scores (dependent variables). Logarithmic (base 10) transformations of numerical data were used where tests of normality (Shapiro Wilk) failed.

We used binary logistic regression to determine the effect of age, disease duration, motor subtype, family history and LEDD (covariates) on the dependent variable i.e. presence or absence of dyskinesia. A smiliar model was used to determine the effect of these covraiates on the other dependent variable i.e presence or absence of dystonia.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (version *23 for Windows*; IL, USA).

6.4 Results

276 patients had completed all questionnaires relevant to this analysis and are included here (Figure 6.1). Their demographic details have been presented in Table 5.1 in Chapter 5.

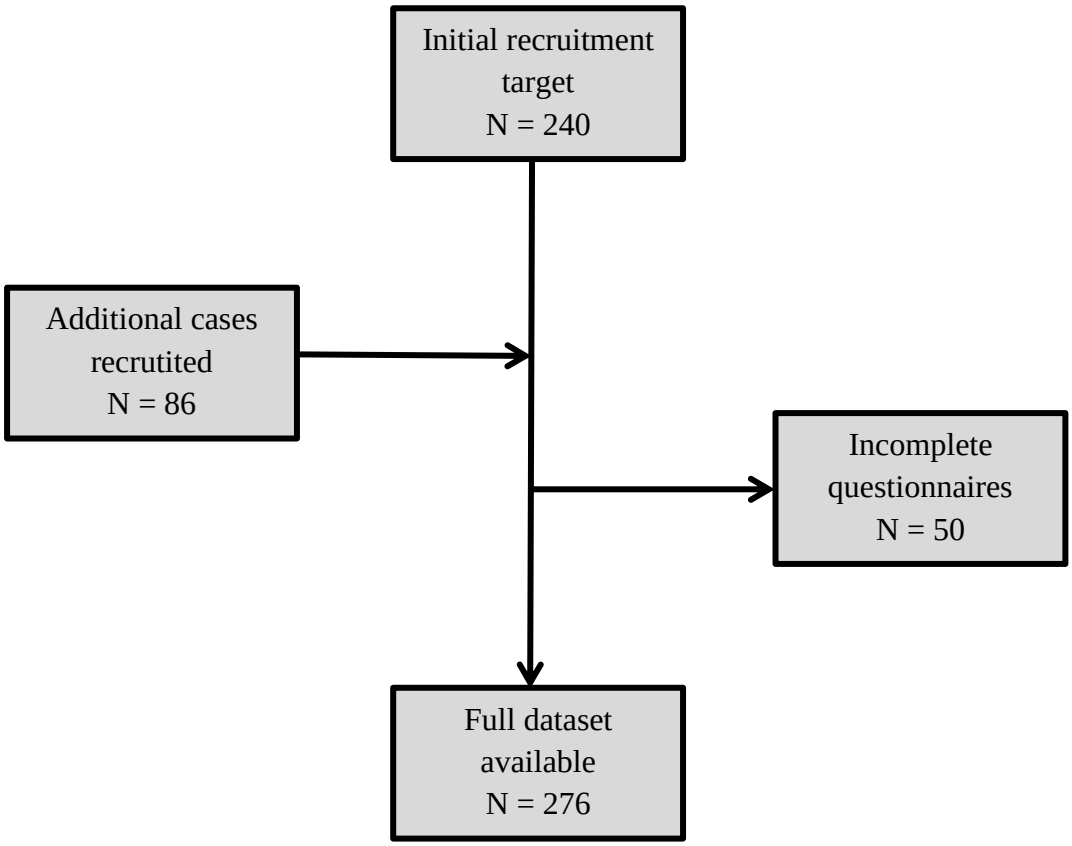

Figure 6.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in the rates of motor complications such as dyskinesia (Figure 6.2) and dystonia (Figure 6.3), medication requirements and UPDRS4 scores in patients, classified by motor subtype, is shown in Table 6.1.

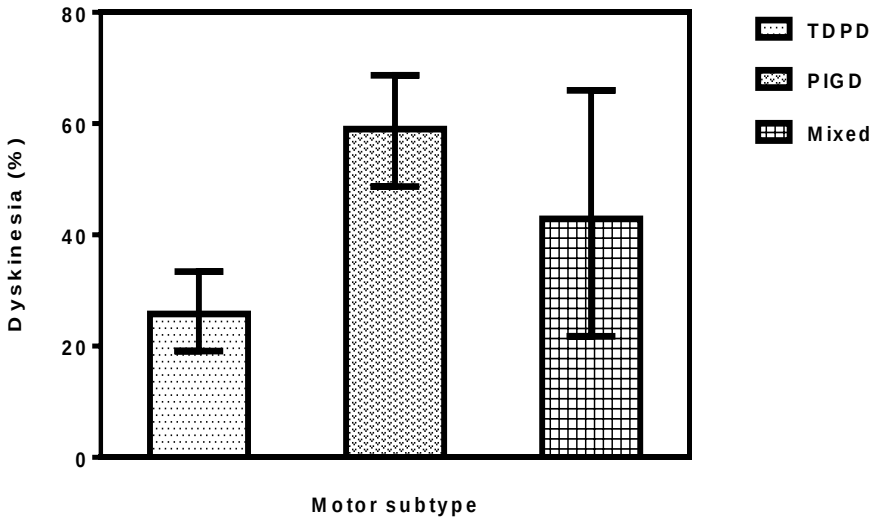

Figure 6.2 shows the proportion of patients who had dyskinesia as recorded on the unified Parkinson's disease rating scale Part 4 (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with dyskinesia, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

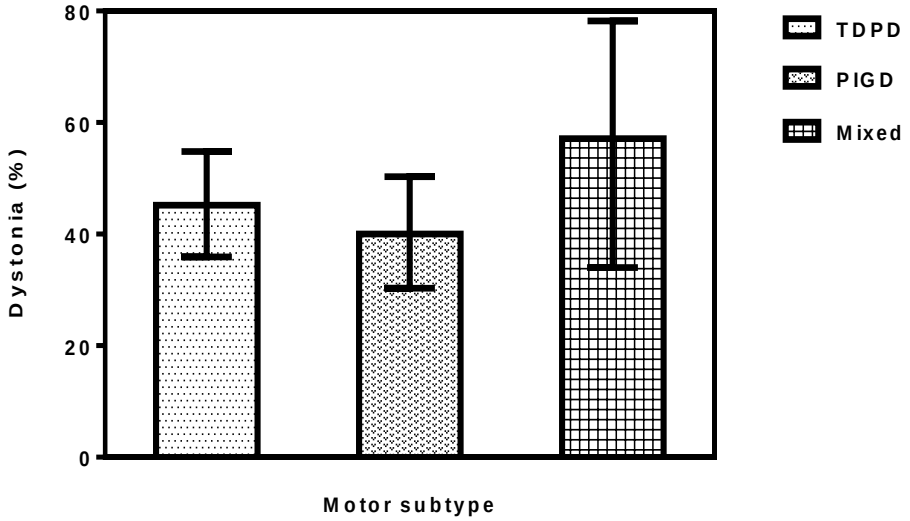

Figure 6.3 shows the proportion of patients who had dystonia as recorded on the unified Parkinson's disease rating scale Part 4 (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with dystonia, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

Table 6.1 Variation in motor complications of Parkinson's disease in patients (n=276) classified by motor subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed subtype** median (IQR)	**p-value**
Number of cases	155	100	21	**-**
Age***** (in years)	50.4 (45.1-54.3)	54.6 (48.8-59.8)	49.6 (46.9-52.5)	**<0.001**
Sex (males)	64.1 %	59.0 %	65.0 %	0.356
Duration† (years)	5.1 (2.0-9.6)	9.1 (4.6-15.0)	7.4 (2.5-11.8)	**<0.001**
Dyskinesia	25.8 %	59.6 %	42.9 %	**<0.001**
On-off fluctuations	47.7 %	66.7 %	52.4 %	**0.016**
Dystonia	33.5 %	40.8 %	57.1 %	0.093
Freezing (s)	41.9 %	74.0 %	52.4 %	**<0.001**
Freezing (o)	12.3 %	47.0 %	28.6 %	**<0.001**
LEU (mg/day)	599 (300-965)	840 (532-1043)	738 (538-1053)	**0.003**
UPDRS4	2 (0-6)	5 (2-10)	4 (0-10)	0.117

*** Age at registration, † disease duration, IQR = inter-quartile range, (s) = subjective from MDS-UPDRS Part 2, (o) = objective from MDS-UPDRS Part 3, LEU = levodopa equivalent untis, UPDRS4 = MDS-UPDRS Part 4 score**

Post hoc calculations showed dyskinesia more prevalent in the PIGD group compared to TDPD ($p<0.001$) but not between TDPD and 'Mixed' ($p=0.121$) or between PIGD and 'Mixed' ($p=0.175$) motor subtypes.

On-off fluctuations were also more prevalent in the PIGD group compared to TDPD ($p=0.004$) but not between TDPD and 'Mixed' ($p=0.817$) or between PIGD and 'Mixed' ($p=0.318$) on post hoc tests.

Freezing as reported subjectively, as recorded on MDS-UPDRS Part 2, was more prevalent in PIGD compared to TDPD ($p<0.001$) but not between TDPD and 'Mixed' ($p=0.482$) or between PIGD and 'Mixed' motor subtypes ($p=0.066$) on post hoc tests. This was replicated in the prevalence of freezing, as recorded objectively, on MDS-UPDRS Part 3 between PIGD and TDPD ($p<0.001$) but not between TDPD and Mixed ($p=0.087$) or between PIGD and 'Mixed' ($p=0.150$) on post hoc tests.

Medication requirements in LEU (mg/day) were highest in the PIGD group compared to TDPD ($p<0.001$) but not between TDPD and Mixed ($p= 0.134$) or between PIGD and 'Mixed' ($p=0.616$) on post hoc tests.

Classifying patients by gender showed that there were no differences between males and females in medication requirements in LEU (mg/day) as shown in Figure 6.4, MDS-UPDRS Part 4 scores (Figure 6.5) or any of the other complications of treatment (Table 6.2).

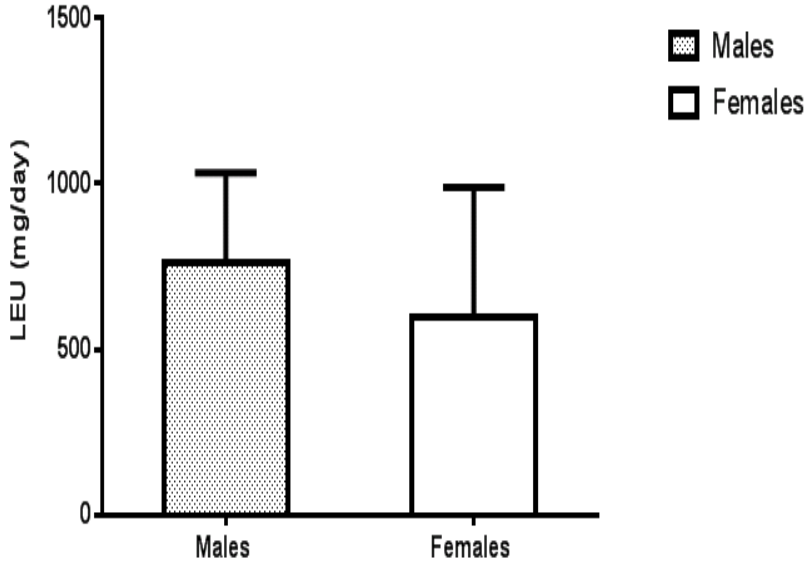

Figure 6.4 shows the medication requirements as levodopa equivalent units (LEU) in mg/day (on the y-axis) and the gender (on the x-axis). No significant differences were found between males (n=177) compared to females (n = 99) ($p=0.136$).

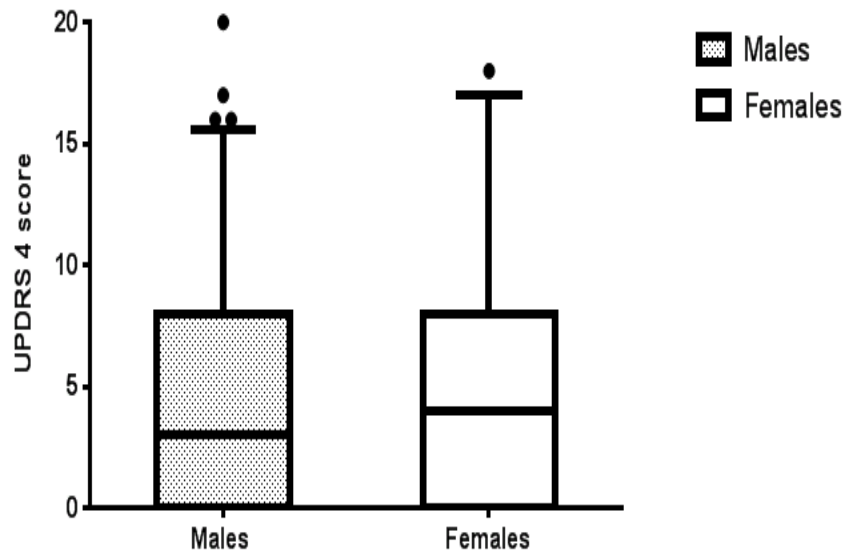

Figure 6.5 shows the unified Parkinson's disease rating scale part 4 scores (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between males (n=177) compared to females (n=99) (p=0.886).

Table 6.2 Variation in motor complications of Parkinson's disease in patients (n=276) classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p- value**
Number of cases	177	99	-
Age ***** (years)	51.7 (46.5-56.8)	51.1 (47.2-55.6)	0.968
Duration† (years)	7.3 (2.8-11.5)	6.5 (2.6-11.9)	0.737
Dyskinesia	37.3 %	42.4 %	0.402
On- Off fluctuations	53.1 %	57.6 %	0.474
Dystonia	37.9 %	37.4 %	0.937
Freezing (subjective)	53.7 %	55.6 %	0.763
Freezing (objective)	27.7 %	23.2 %	0.419
LEU (mg/day)	760 (400-1030)	600 (328-986)	0.136
UPDRS 4	3 (0-8)	4 (0-8)	0.886

*** age at registration, † disease duration, IQR = inter-quartile range, (s) = subjective from MDS-UPDRS Part 2, (o) = objective from MDS-UPDRS Part 3, LEU= levodopa equivalent untis, UPDRS4= MDS-UPDRS Part 4 score**

There were also no differences between those diagnosed with PD aged less than (or equal to) of 40 years compared to those diagnosed after the age of 40 years in the EOPD cohort in medication requirements recorded in LEU (mg/day) as shown in Figure 6.6, MDS-UPDRS Part 4 scores (Figure 6.7) or any of the other complications of treatment (Table 6.3).

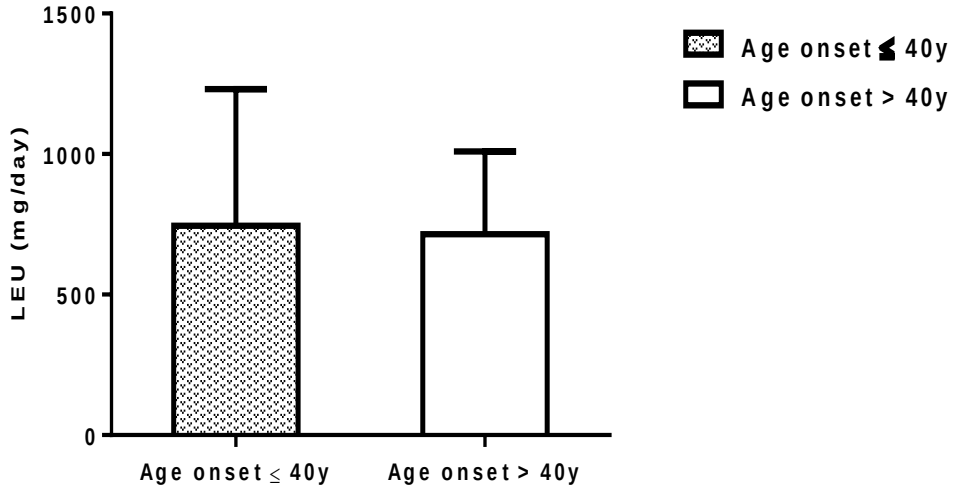

Figure 6.6 shows the medication requirements as levodopa equivalent units (LEU) in mg/day (on the y-axis) and the age at onset in years (on the x-axis). No significant differences were found between those with age at onset of PD ≤ 40 years (n=64) compared to those with age at onset of PD > 40 years (n=212) (p=0.659).

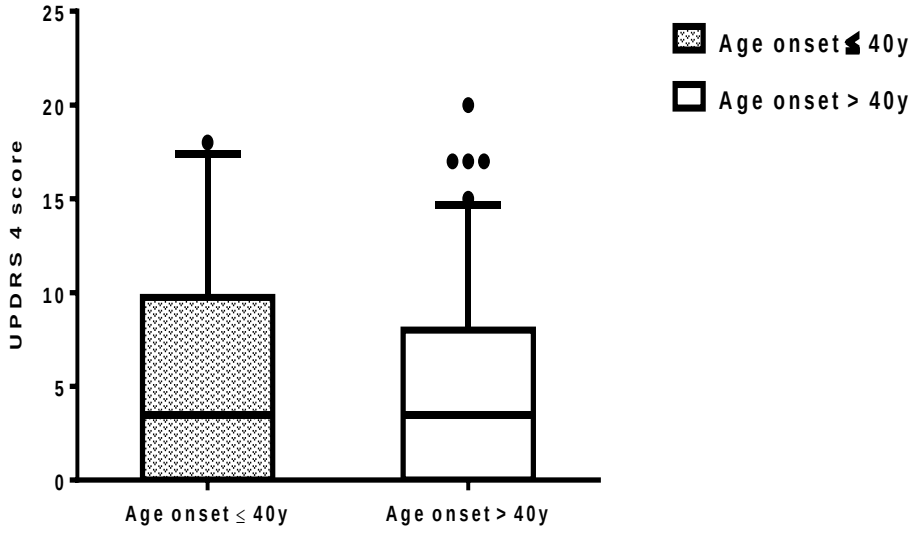

Figure 6.7 shows the unified Parkinson's disease rating scale part 4 scores (on the y-axis) and the age at onset in years (on the x-axis. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with age at onset of PD ≤ 40 years (n=64) compared to those with age at onset of PD > 40 years (n=212) (p=0.275).

Table 6.3 Variation in motor complications of Parkinson's disease in patients (n=276) classified by age at onset.

Variable	**Age at onset ≤ 40 years** median(IQR)	**Age at onset > 40 years** median(IQR)	**p- value**
Number of cases	64	212	-
Age***** (years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	<0.001
Sex (males)	67.2%	63.2%	0.561
Duration† (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.707
Dyskinesia	20.0 %	39.9 %	0.148
On-off fluctuations	40.0 %	55.1 %	0.564
Dystonia	33.3 %	37.6 %	0.579
Freezing (subjective)	40.0 %	54.8 %	0.357
Freezing (objective)	6.7 %	27.0 %	0.283
LEU (mg/day)	700 (363-998)	715 (400-1008)	0.659
UPDRS4	4 (0-9)	3 (0-8)	0.275

*** age at registration, † disease duration, IQR= interquartile range. Data are presented in median (inter-quartile range) except where indicated, (s) = subjective from MDS-UPDRS Part 2, (o) = objective from MDS-UPDRS Part 3, LEU= levodopa equivalent units, UPDRS4= MDS-UPDRS Part 4 score**

The only difference in the motor complications between those with a positive family history of PD and those with a negative family history of PD was the prevalence of dystonia being higher in those in the former category (p=0.020) as shown in Table 6.4. There was no difference in the medication requirements (Figure 6.8), MDS-UPDRS Part 4 scores (Figure 6.9) or any of the other motor complications of PD between the 2 groups (Table 6.4).

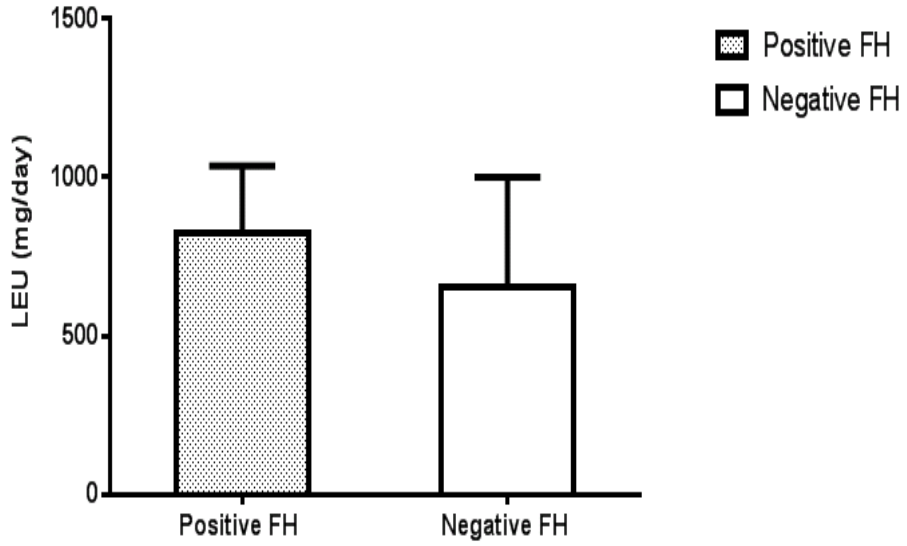

Figure 6.8 shows the medication requirements as levodopa equivalent units (LEU) in mg/day (on the y-axis) and the family history (FH) of Parkinson's disease (on the x-axis). No significant differences were found between those with familial PD (n=64) compared to those with sporadic PD (n=212) (p=0.135).

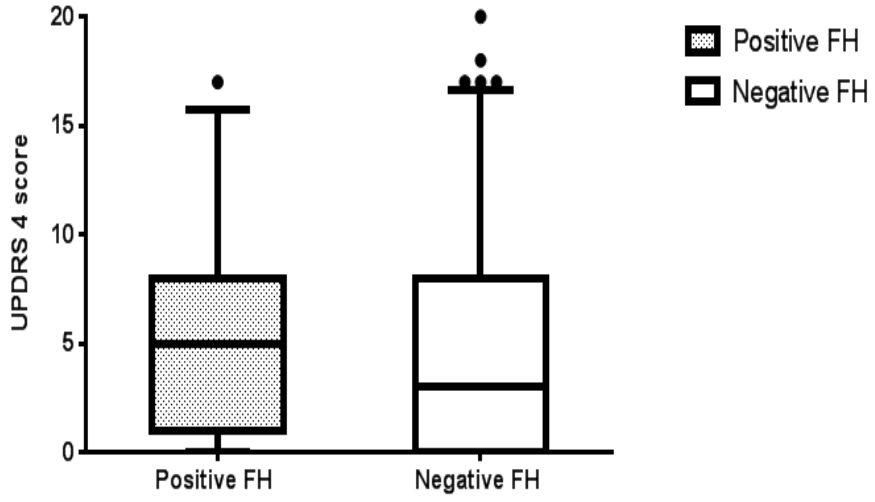

Figure 6.9 shows the unified Parkinson's disease rating scale part 4 scores (on the y-axis) and the family history (FH) of Parkinson's disease (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with familial PD (n=64) compared to those with sporadic PD (n=212) (p=0.785).

Table 6.4 Variation in motor complications of Parkinson's disease in patients (n=276) classified by family history.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p- value**
Number of cases	64	212	-
Age***** (years)	52.2 (47.3-56.8)	51.2 (46.9-56.1)	0.582
Sex (males)	65.6 %	63.7 %	0.776
Duration (years)	6.7 (3.5-12.1)	6.8 (2.5-11.3)	0.312
Dyskinesia	47.0 %	36.3 %	0.082
On- Off fluctuations	54.5 %	54.2 %	0.777
Dystonia	48.5 %	34.0 %	**0.020**
Freezing (subjective)	53.0 %	54.5 %	0.979
Freezing (objective)	18.2 %	28.3 %	0.127
LEU (mg/day)	824 (460-1033)	655 (400-1000)	0.135
UPDRS 4	5 (1-8)	3 (0-8)	0.785

FH= family history, * Age at registration, † disease duration, IQR = inter-quartile range. Data are presented in median (inter-quartile range) except where indicated, (s) = subjective from MDS-UPDRS Part 2, (o) = objective from MDS-UPDRS Part 3, LEDD = levodopa equivalent units, UPDRS4 = MDS-UPDRS Part 4 score

Binary logistic regression analyses showed that the only variables that had a significant effect on the presence or absence of dyskinesia were disease duration ($p<0.001$), the PIGD motor subtype ($p=0.009$) and the HY stage ($p=0.016$). All the other covraiates tested in this model i.e age ($p=0.244$), gender ($p=0.304$), family history ($p=0.107$) and LEDD ($p=0.350$) had no significant effect on the presence of dyskinesia.

Using analogous binary logistic regression analyses, the only variables that had a significant effect on the presence or absence of dystonia were the Mixed motor subtype ($p=0.013$) and the HY stage ($p=0.010$). All the other covraiates tested in this model i.e age ($p=0.543$),

disease duration (p=0.682), gender (p=0.611), family history (p=0.073) and LEDD (p=0.342) had no significant effect on the presence of dystonia.

6.5 Discussion:

The development of motor complications in approximately half of patients treated with levodopa after 5-6 years had been recognised in the early years after the introduction of this drug as standard therapy for treating parkinsonian symptoms [160] but the development of motor fluctuations is also influenced by the total daily dose of levodopa. The proportion of patients affected with motor fluctuations at 5 years could be as low as 20 % with prescription of lower total daily doses of levodopa [342].

The relationship of motor subtypes to the prevalence of developing motor complications has been reported before. Multivariate logistic regression analysis demonstrated that TDPD was associated with a reduced risk of levodopa induced dyskinesia, independent of other risk factors, such as age at the onset of PD, the duration and dose of levodopa [343]. We also found that motor complications such as dyskinesia ($p<0.001$), 'on' 'off' fluctuations ($p= 0.004$) and freezing ($p<0.001$) were more prevalent in the PIGD group compared to the TDPD group however there were no significant differences between the Mixed subtype and TDPD or PIGD. This would not be surprising if one considers that TDPD and PIGD represent ends of a spectrum with ‘Mixed’ representing a sub-group that has features of both and can be imagined to lie somewhere in the middle of that spectrum.

Some have argued that postural instability with falling (PIF) and freezing of gait (FOG) are distinct subtypes within the postural PIGD group [344]. While this remains a point of debate what is clear is that FOG is most prevalent in the PIGD motor subtype compared to TDPD and ‘Mixed’. The following four models have been proposed to underlie the episodic nature of FOG: (1) The threshold model assumes that FOG occurs when the accumulation of various motor deficits reinforce each other to a point of motor breakdown; (2) the interference model proposes that FOG represents an inability to deal with concurrent cognitive, limbic, and motor input, causing an interruption of locomotion; (3) the cognitive model views FOG as induced by a failure to process response conflict, leading to behavioural indecision; and (4) the decoupling model sees FOG as a disconnection between preparatory programming and

the intended motor response as a result of which automatic movement generation gets stuck [345], it is probably a mix of these all hypothetical model that will explain the variation in FOG with motor subtype. Cases with the PIGD motor subtype have the greatest motor problems, disability (as shown in Chapter 5) and cognitive impairment (as shown in Chapter 8) , after correcting for the effects of age and disease duration, compared to TDPD and 'Mixed' phenotypes. Therefore to summarise it is probably the depletion of motor and cognitive reserves and an increasingly complex response to levodopa with disease progression that will leads to the emergence of FOG [345].

Of all the motor complications dyskinesia is reported to be the most common [346]. Our study however, showed that FOG, as recorded objectively on MDS-UPDRS Part 3, was the most common documented complication in the whole EOPD cohort (73.9%) followed by dystonia (62.3%), dyskinesia (60.9%) and 'on' 'off' fluctuation (45.3%), as documented on MDS-UPDRS Part 3 . This has obviously to be interpreted in the light of disease duration of this cohort which was 6.5 years (median), inter-quartile range 2.6-11.9 years, and medication usage in LEDD 682.5mg/day (median), inter-quartile range 368.8-1000.0 mg/day.

There are gender differences in the development of peak dose dyskinesia. Multivariate logistic regression analysis was used to show that independent predictors for the occurrence of peak dose dyskinesia besides higher dose of levodopa and longer duration of treatment were female sex and earlier age at onset of PD [347]. We did not find any statistically significant differences in the prevalence of dyskinesia (p= 0.402) or any of the motor complications subjectively or objectively between the genders.

The age at onset can influence the rate of motor complications in PD. Patients with a younger age at onset are reported to exhibit higher risk of dyskinesia or dystonia compared to older age of PD onset [339], however, our results didn't show any differences in the rates of either dyskinesia (p=0.148) or dystonia (p=0.579) between those diagnosed with PD before the age of 40 years compared to those diagnosed after the age of 40 years. This is probably due to the fact that the population analysed in Wickremaratchi et al's study had a different demographic profile including a mix of EOPD and LOPD cases whereas our study cohort was more homogeneous as it included only EOPD cases. The mean age at assessment in that study compared to the cohort analysed here was 65 years vs. 51.5 years, mean age at onset 56

years vs. 43.5 years, mean disease duration was 9 years vs. 7.9 years and mean motor UPDRS Part 3 score was 28 vs. 26.

We found dystonia to be more prevalent in those with a family history of PD compared to those with sporadic PD (P=0.020). Patients with *Parkin* mutations more commonly present with dystonia and have a positive family history of PD [348]. This can be a possible explanation for the statistically significant differences in the prevalence of dystonia between the 2 sub-groups given that medication usage in LEDD was not significantly different.

6.6 Conclusion

Factors that are responsible for the variation in the prevalence of motor complications of PD include duration of disease, duration of levodopa treatment, cumulative and levodopa daily dose, earlier age at onset of disease, female sex, and possibly genetic factors but differences in the methods used to assess the presence of these complications (self-reporting versus objective assessment), the setting (hospital or community based clinic versus research protocol) and different patient populations may also contribute to some of the variation in reported prevalence rates.

Chapter 7. Variation in non-motor symptoms in EOPD

7.1 Objective

The objective in this chapter is to analyse the variation in the non-motor symptoms in a cohort of patients with early onset Parkinson's disease. Olfaction data will be analysed and presented separately in Chapter 8.

7.2 Introduction

PD has traditionally been viewed as a motor syndrome arising from nigrostriatal dopaminergic denervation, however, there is sufficient evidence to indicate that PD is a multisystem neurodegenerative disorder with dozens of non-motor symptoms [349]. The recognition of non-motor symptoms (NMS) in PD is important not only because these are widely prevalent but also because NMS have a direct negative impact on health-related and perceived quality of life in PD, sometimes this effect can be larger than that due to the motor disability in these patients [350].

7.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0), physical examination to record changes in blood pressure with posture as a measure of orthostatic hypotension was recorded at the same visit. Data required for documenting other features of autonomic dysfunction including orthostatic symptoms, gastrointestinal, genitourinary, sudomotor, temperature intolerance were collected from patient filled validated questionnaires such as: scales for outcomes in PD-autonomic (SCOPA-AUT) [72] , gastroparesis cardinal symptom index (GCSI) [351] and non-motor symptom assessment scale for Parkinson's disease (NMSS) [352] at visit 1(0-6 months). The prevalence of restless

legs symptoms, unexplained pain and fatigue as significant non-motor symptoms that impact on the perceived quality of life by patients [353, 354] were also recorded from the NMSS questionnaire.

Excessive day time sleepiness, rapid eye movement sleep behaviour disorder (RBD) symptoms and sleep dysfunction were also recorded at visit 1 using 3 validated instruments Epworth sleepiness scale (ESS) [355], Parkinson's disease sleep scale (PDSS) [356] and Rapid eye movement (REM) sleep behaviour disorder (RBD) questionnaire [357].

In order to analyse variation in the non-motor phenotype of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed'), gender, age at onset of PD symptoms and family history of PD as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data, with post-hoc tests as appropriate.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (*version 23 for Windows*; IL, USA).

7.4 Results

276 patients had completed all questionnaires relevant to this analysis and are included here (Figure 7.1). Their demographic details have been presented in Table 5.1 in Chapter 5.

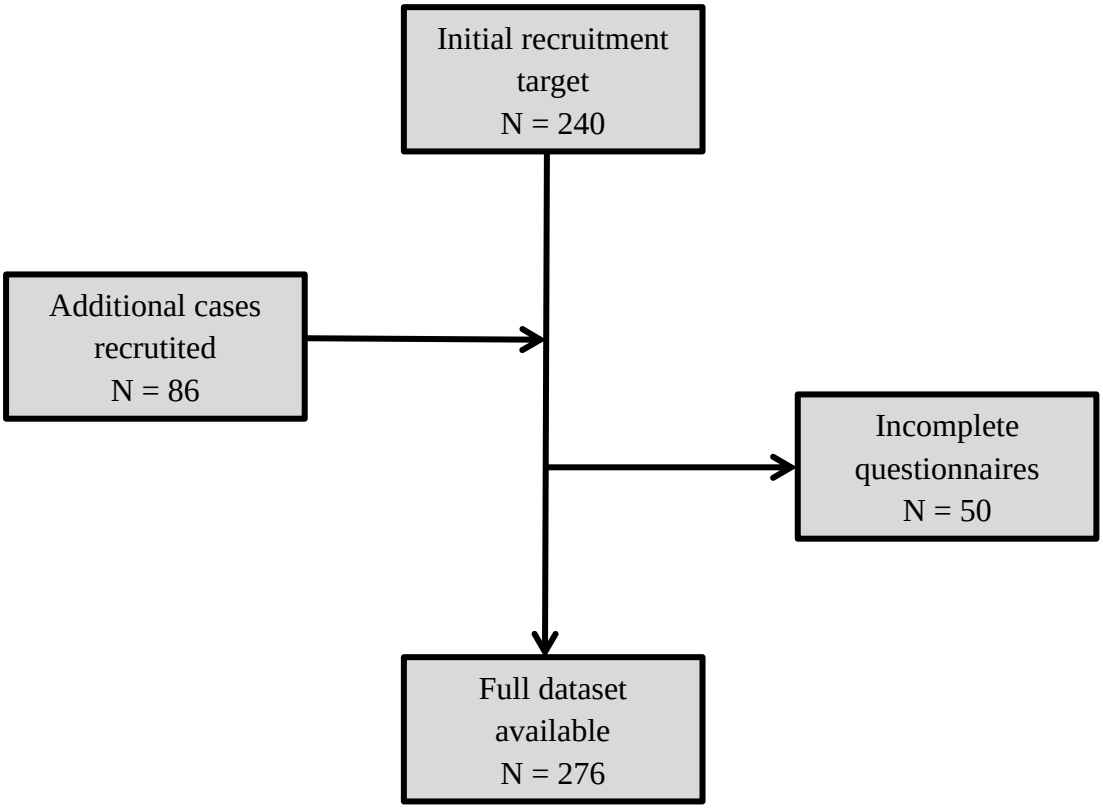

Figure 7.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in the prevalence of non-motor symptoms in these patients, classified by motor subtype, is presented in Tables 7.1a and 7.1b. There were no statistically significant differences in the prevalence of autonomic (Figure 7.2), gastrointestinal (Figure 7.3), genitourinary, sleep dysfunction (Figures 7.4 to 7.6), fatigue or pain symptoms between the three motor subtypes (Tables 7.1a and 7.1b)

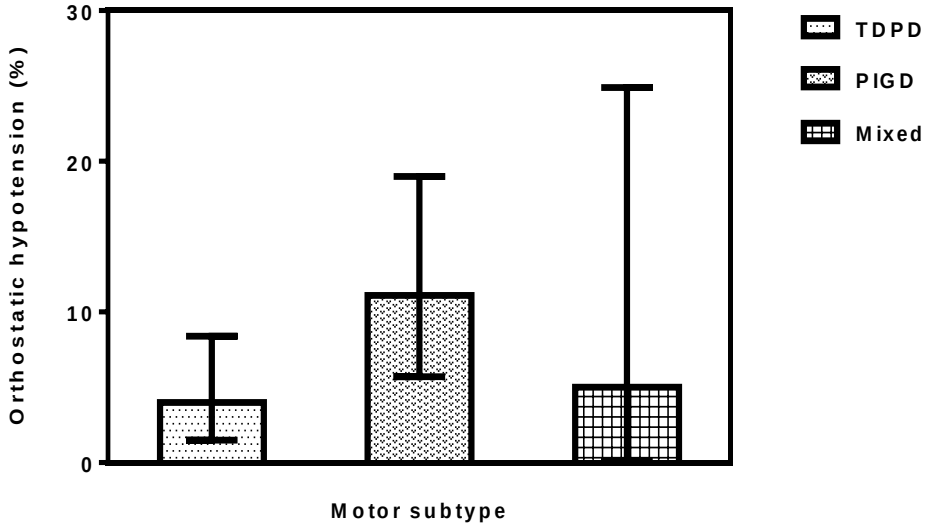

Figure 7.2 shows the proportion of patients who had orthostatic hypotension on examination, defined as a drop of 20 mm Hg in the systolic blood pressure and/or 10 mm Hg in the diastolic blood pressure within 3 minutes of standing from a lying position (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with orthostatic hypotension, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

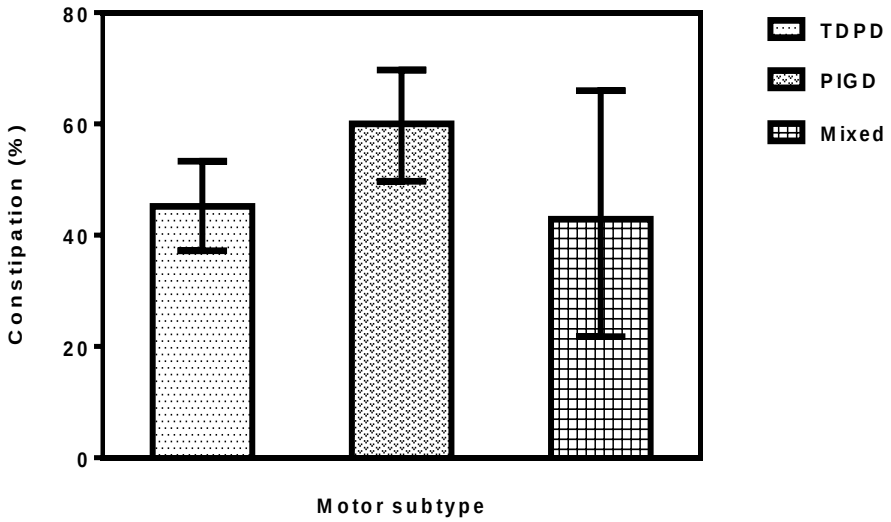

Figure 7.3 shows the proportion of patients who reported constipation, as recorded on the SCOPA-AUT questionnaire (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases who reported constipation, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

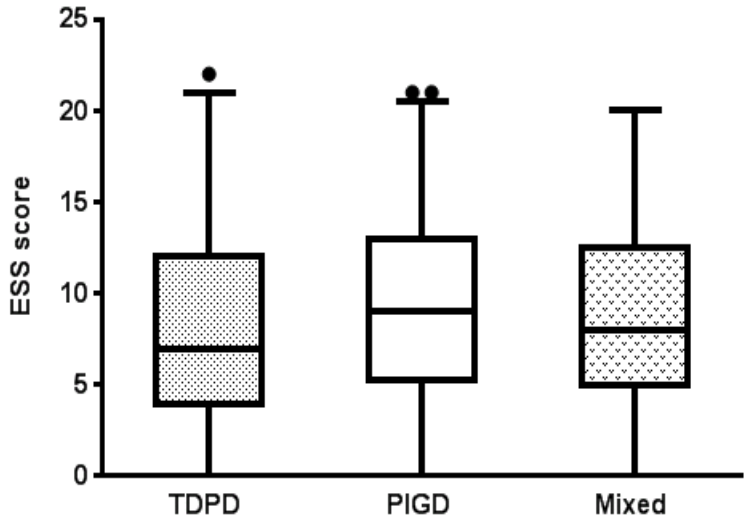

Figure 7.4 shows the Epworth sleepiness scale score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between thoses with TDPD (n=155), PIGD (n=100) or 'Mixed' (n=21) motor subtype (p=0.064).

Table 7.1a Variation in non-motor symptoms in patients (n=276) classified by motor subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number of cases	155	100	21	
Age* (in years)	50.4 (45.1-54.3)	54.6 (48.8-59.8)	49.6 (46.9-52.5)	**<0.001**
Sex (males)	67.7 %	59.0 %	65 %	0.356
Duration (years)	5.1 (2.0-9.6)	9.1 (4.6-15.0)	7.4 (2.5-11.8)	**<0.001**
Autonomic symptoms				
Orthostatic symptoms	47.1 %	54.0 %	61.9 %	0.317
Orthostatic hypotension†	4.0 %	11.1 %	5.0 %	0.082
Hyperhidrosis (day)	45.8 %	45.0 %	42.9 %	0.965
Hyperhidrosis (night)	51.6 %	56.0 %	47.6 %	0.696
Cold intolerance	50.3 %	50.0 %	61.9 %	0.587
Heat intolerance	47.7 %	47.0 %	47.6 %	0.993
Gastrointestinal symptoms				
Dysphagia	31.6 %	38.0 %	23.8 %	0.360
Prandial bloating	30.3 %	30.0 %	28.6 %	0.986
Constipation	45.2 %	60.0 %	42.9 %	0.053
Incontinence (faecal)	7.1 %	9.0 %	0 %	0.349

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, * Age at registration, IQR= inter-quartile range, †orthostatic hypotension was defined as fall in systolic blood pressure of 20 mm Hg or more or diastolic blood pressure of 10 mm Hg or more on standing.

There were no statistically significant differences in the NMSS total scores between the three motor subtypes (p=0.155) as shown in Table 7.1b and figure 7.7.

Table 7.1b Variation in non-motor symptoms in patients (n=276) classified by motor subtype (continuation of Table 7.1a)

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number of cases	155	100	21	-
Genitourinary dysfunction				
Bladder dysfunction*	93.5 %	98.0 %	100.0 %	0.140
Erectile dysfunction (m)	46.7 %	50.8 %	38.5 %	0.697
Anorgasmia (f)	32.0 %	51.2 %	50.0 %	0.158
Incontinence (urinary)	34.8 %	40.0 %	23.8 %	0.343
Sleep dysfunction				
RBD† symptoms	36.8 %	46.0 %	38.1 %	0.334
Disrupted sleep‡	51.7 %	38.4 %	55.0 %	0.092
EDS§	32.3 %	40.0 %	33.3 %	0.442
Restless legs symptoms	65.6 %	73.7 %	55.0 %	0.179
Pain (unexplained)	35.8 %	46.5 %	45.0 %	0.129
Fatigue	76.8 %	80.8 %	85.0 %	0.590
NMSS total score	36 (21-65)	48 (25-77)	43 (26-84)	0.155

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, *Bladder dysfunction= difficulty retaining urine, feeling bladder not completely empty, weak stream, pis en deux or nocturia, (m) = males, (f) = females. RBD = Rapid eye movement sleep behaviour disorder, †RBD questionnaire score ≥ 5, ‡ using Parkinson's disease sleep scale (PDSS) score > 100, § EDS = excessive daytime sleepiness using Epworth sleep scale (ESS) score ≥ 11, NMSS = non-motor symptom scale.

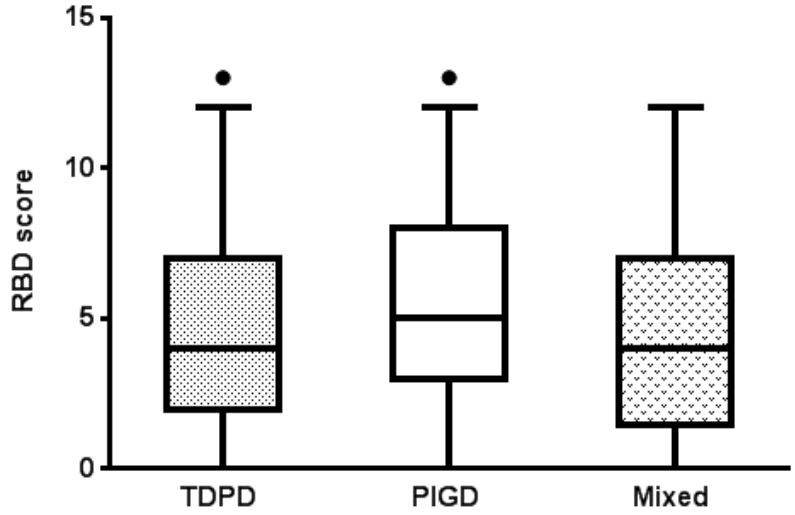

Figure 7.5 shows the REM sleep behaviour disorder scale score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between thoses with TDPD (n=155), PIGD (n=100) or 'Mixed' (n=21) motor subtype (p=0.256).

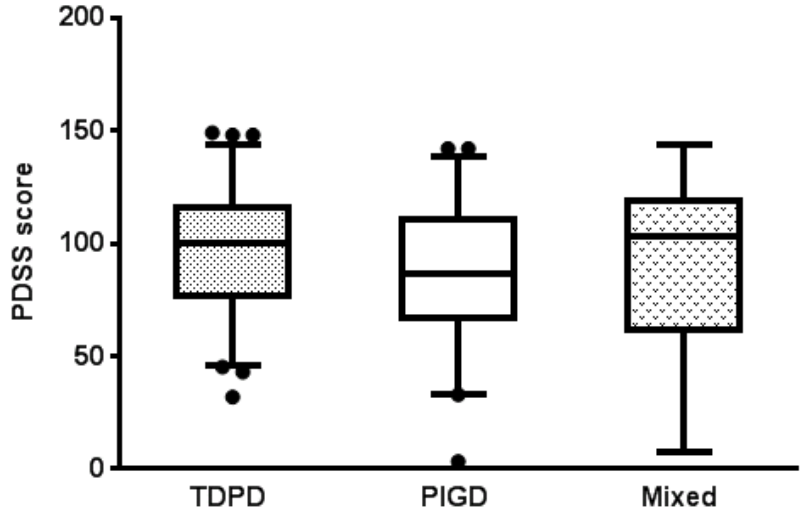

Figure 7.6 shows the Parkinson's disease sleep scale score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. PDSS scores were significantly greater in those with TDPD (n=155) compared to PIGD (n=100) (p=0.031).

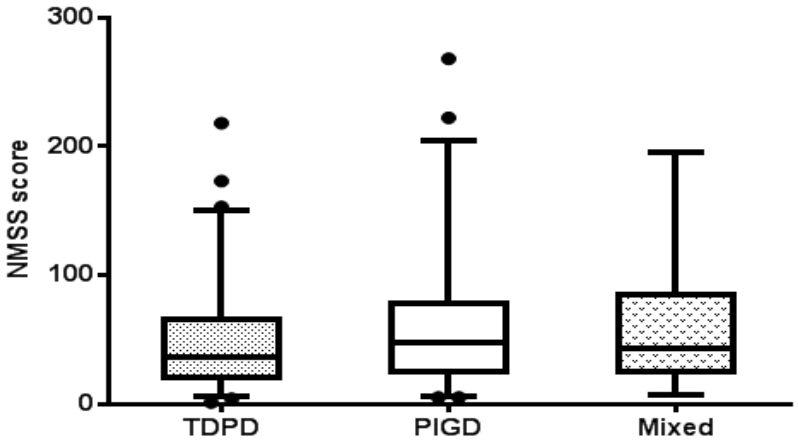

Figure 7.7 shows the non-motor symptoms scale score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the NMSS total scores were found between thoses with TDPD (n=155), PIGD (n=100) or 'Mixed' (n=21) motor subtype (p=0.155).

The prevalence of some gastrointestinal, genitourinary and pain symptoms showed gender differences as shown in Table 7.2a and 7.2b. Both bladder and bowel incontinence (Figure 7.8) were more commonly reported by females.

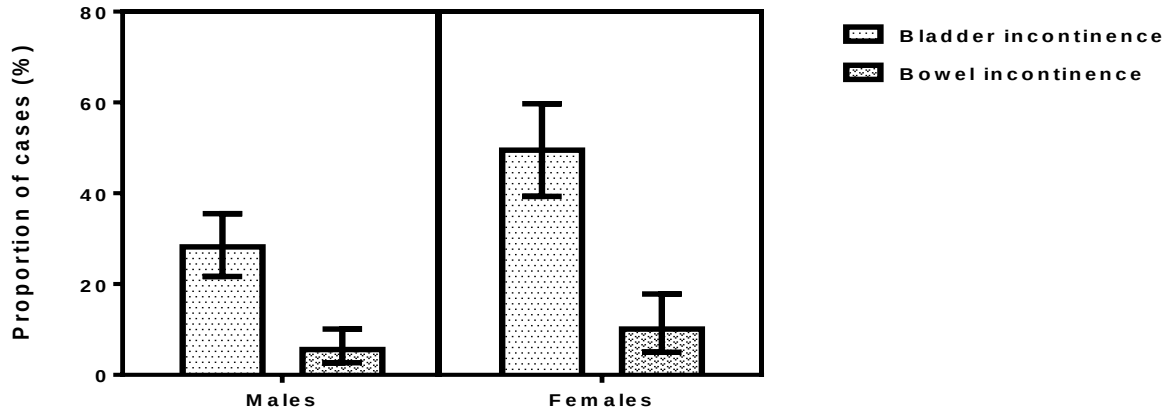

Figure 7.8 shows the proportion of patients who reported bladder or bowel incontinence, as recorded on the SCOPA-AUT questionnaire (on the y-axis) and their gender (on the x-axis).

Table 7.2a Variation in non-motor symptoms in patients (n=276) classified by gender

Variable	**Males** median (IQR)	**Females** median (IQR)	**p- value**
Number of cases	177	99	-
Age* (years)	51.7 (46.5-56.8)	51.1 (47.2-55.6)	0.968
Disease duration (years)	7.3 (2.8-11.5)	6.5 (2.6-11.9)	0.737
Autonomic symptoms			
Orthostatic symptoms	48.6 %	54.5 %	0.342
Orthostatic hypotension†	7.3 %	5.1 %	0.613
Hyperhidrosis (day)	43.5 %	48.5 %	0.425
Hyperhidrosis (night)	48.6 %	60.6 %	0.056
Cold intolerance	49.7 %	53.5 %	0.543
Heat intolerance	45.8 %	50.5 %	0.450
Gastrointestinal symptoms			
Dysphagia	39.5 %	22.2 %	**0.003**
Prandial bloating	25.4 %	38.4 %	**0.024**
Constipation	45.2 %	59.6 %	**0.022**
Incontinence (faecal)	5.6 %	10.1 %	0.226

*** Age at registration, IQR= inter-quartile range, † orthostatic hypotension defined as fall in systolic blood pressure of 20 mm Hg or more or diastolic blood pressure of 10 mm Hg or more on standing**

There were no statistically significant differences in the NMSS total scores between the two genders (p=0.743) as shown in Table 7.2b and figure 7.9.

Table 7.2b Variation in non-motor symptoms in patients (n=276) classified by gender (continuation of Table 7.2a)

Variable	**Males** median (IQR)	**Females** median (IQR)	**p- value**
Number of cases	177	99	-
Genitourinary dysfunction			
Bladder dysfunction ¶	96.0 %	94.9 %	0.669
Erectile dysfunction	52.5 %	NA	-
Anorgasmia (females)	NA	65.7 %	-
Incontinence (urinary)	28.2 %	49.5 %	**<0.001**
Sleep dysfunction			
RBD** symptoms	41.2 %	38.4 %	0.642
Disrupted sleep‡	47.5 %	43.4 %	0.520
EDS §	37.9 %	30.3 %	0.208
Restless legs symptoms	64.4 %	69.7 %	0.373
Pain (unexplained)	34.5 %	48.5 %	**0.022**
Fatigue	75.7 %	79.8 %	0.437
NMSS total score	41 (24-70)	36 (24-73)	0.743

IQR = inter-quartile range, ¶ Bladder dysfunction= difficulty retaining urine, feeling bladder not completely empty, weak stream, pis en deux or nocturia. M= males, F= females. RBD = Rapid eye movement behaviour disorder, **RBD questionnaire score ≥ 5, ‡ using Parkinson's disease sleep scale (PDSS) score >100, § EDS= excessive daytime sleepiness using Epworth sleep scale (ESS) score ≥ 11, NMSS = non-motor symptom scale.

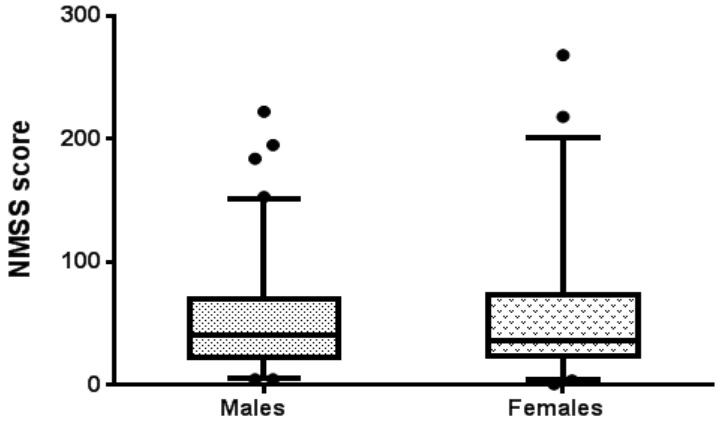

Figure 7.9 shows the non-motor symptoms scale score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the NMSS total scores were found between males (n=177) and females (n=99) (p=0.743).

There were no differences in most of the non-motor symptoms between those diagnosed with Parkinson's disease aged less than (or equal to) 40 years compared to those diagnosed after the age of 40 years as shown in Tables 7.3a and 7.3b including sexual dysfunction (Figure 7.10).

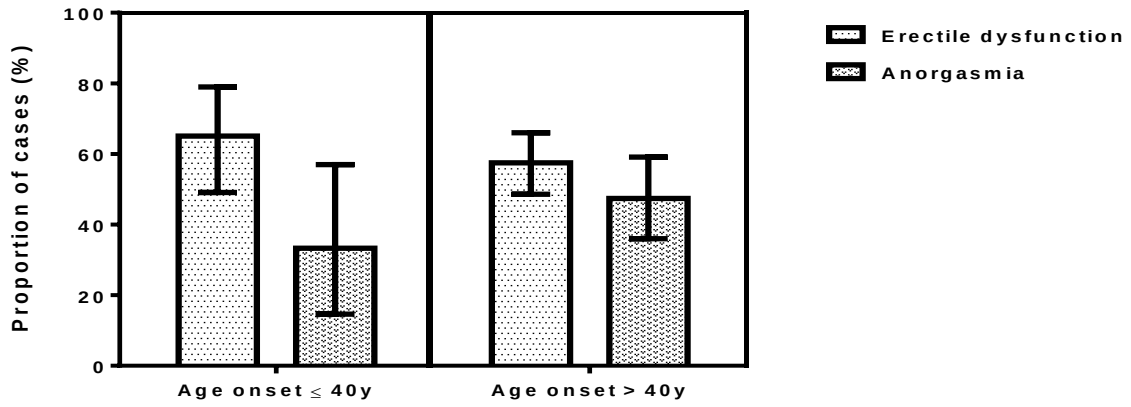

Figure 7.10 shows the proportion of patients who reported erectile dysfunction or anorgasmia, as recorded on the SCOPA-AUT questionnaire (on the y-axis) and their age at diagnosis of PD (on the x-axis).

Table 7.3a Variation in non-motor symptoms in patients (n=276) classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40 yrs.** median (IQR)	**p-value**
Number of cases	64	212	-
Age***** (in years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	**<0.001**
Sex (males)	67.2 %	63.2 %	0.561
Disease duration (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.707
Autonomic symptoms			
Orthostatic symptoms	57.8 %	48.1 %	0.196
Orthostatic hypotension ¶	9.5 %	5.7 %	0.385
Hyperhidrosis (day)	45.3 %	45.3 %	0.997
Hyperhidrosis (night)	57.8 %	51.4 %	0.369
Cold intolerance	54.7 %	50.5 %	0.511
Heat intolerance	51.6 %	46.3 %	0.454
Gastrointestinal symptoms			
Dysphagia	40.6 %	30.8 %	0.158
Prandial bloating	29.7 %	29.9 %	0.939
Constipation	42.2 %	52.8 %	0.136
Incontinence (faecal)	6.3 %	7.5 %	0.726

*** Age at registration, IQR = interquartile range, ¶ orthostatic hypotension defined as fall in systolic blood pressure of 20 mm Hg or more or diastolic blood pressure of 10 mm Hg or more on standing.**

There were no statistically significant differences in the NMSS total scores between the two groups (p=0.227) as shown in Table 7.3b and Figure 7.11.

Table 7.3b Variation in non-motor symptoms in patients (n=276) classified by age at onset of PD (continuation of Table 7.3a)

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40 yrs.** median (IQR)	**p-value**
Number of cases	64	212	-
Genitourinary dysfunction			
Bladder dysfunction †	93.8 %	96.3 %	0.395
Erectile dysfunction(m)	65.1 %	57.5 %	0.374
Anorgasmia (f)	33.3 %	47.4 %	0.248
Incontinence (urinary)	29.7 %	37.4 %	0.240
Sleep dysfunction			
RBD**symptoms	40.6 %	40.1 %	0.940
Disrupted sleep‡	49.2 %	46.4 %	0.694
EDS §	40.6 %	33.5 %	0.295
Restless legs symptoms	74.6 %	65.7 %	0.186
Pain(unexplained)	44.4 %	39.1 %	0.452
Fatigue	76.2 %	79.7 %	0.549
NMSS total score	45 (26-74)	37 (23-70)	0.227

† Bladder dysfunction = difficulty retaining urine, feeling bladder not completely empty, weak stream, pis en deux or nocturia. (m) = males, (f) = females. RBD = Rapid eye movement behaviour disorder, **RBD questionnaire score ≥ 5, ‡ using Parkinson's disease sleep scale (PDSS) score > 100, § EDS = excessive daytime sleepiness using Epworth sleep scale (ESS) score ≥ 11, NMSS = non-motor symptom scale.

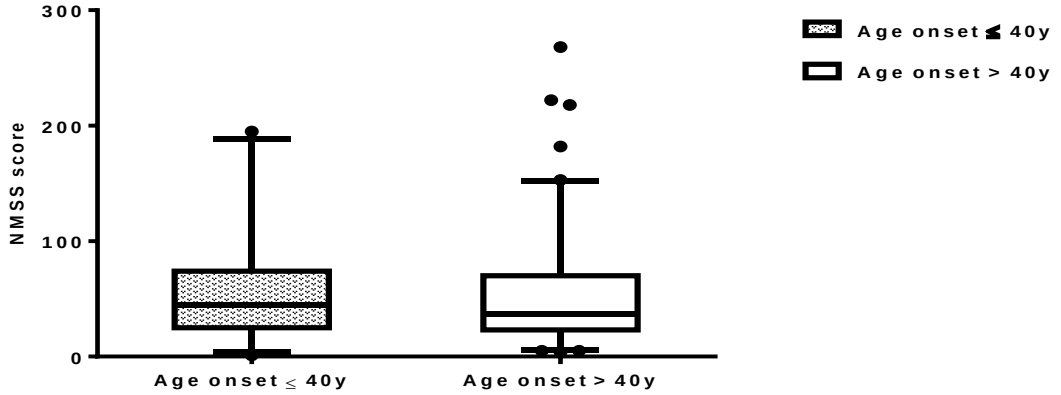

Figure 7.11 shows the non-motor symptoms scale (NMSS) score (on the y-axis) and age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the NMSS total scores were found between those with age at onset of PD ≤ 40 years (n=64) compared to those with age at onset of PD > 40 years (n=212) (p=0.227).

Classifying patients into those with a positive family history of PD and those with a negative family history of PD also showed no differences in most non-motor symptoms except one autonomic symptom i.e. prandial bloating (Figure 7.12) which was more commonly reported by those with a positive family history (p= 0.036) as shown in Table 7.4a and 7.4b.

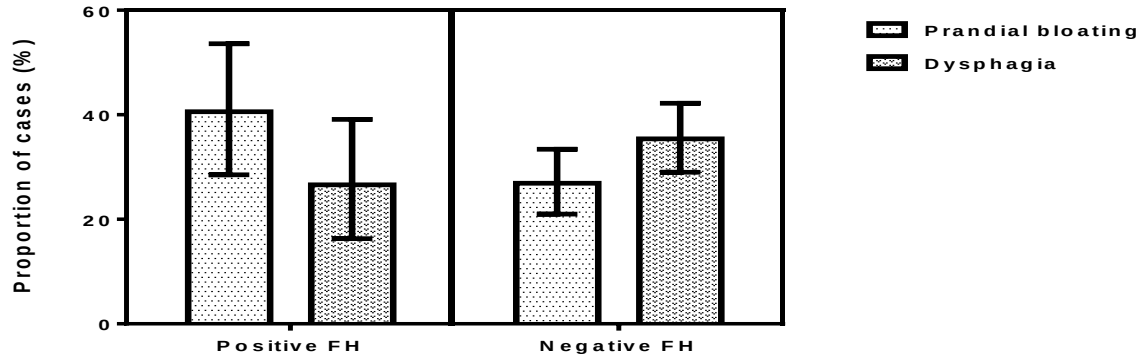

Figure 7.12 shows the proportion of patients who reported prandial bloating and dysphagia, as recorded on the SCOPA-AUT questionnaire (on the y-axis) and their family history (FH) of PD (on the x-axis).

Table 7.4a Variation in non-motor symptoms in patients (n=276) classified by family history of PD.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	64	212	-
Age***** (years)	52.2 (47.3-56.8)	51.2 (46.9-56.1)	0.582
Sex (males)	65.6 %	63.7 %	0.776
Disease duration (years)	6.7 (3.5-12.1)	6.8 (2.5-11.3)	0.312
Autonomic symptoms			
Orthostatic symptoms	51.5 %	50.0 %	0.661
Orthostatic hypotension ¶	6.1 %	1.9 %	0.087
Hyperhidrosis (day)	47.0 %	44.8 %	0.771
Hyperhidrosis (night)	50.0 %	53.8 %	0.597
Cold intolerance	50.0 %	51.9 %	0.629
Heat intolerance	43.9 %	48.6 %	0.497
Gastrointestinal symptoms			
Dysphagia	25.8 %	35.4 %	0.190
Prandial bloating	39.4 %	26.9 %	**0.036**
Constipation	45.5 %	51.9 %	0.357
Incontinence (faecal)	6.1 %	7.5 %	0.726

FH = family history, * age at registration, IQR = inter-quartile range. ¶ orthostatic hypotension defined as fall in systolic blood pressure of 20 mm Hg or more or diastolic blood pressure of 10 mm Hg or more on standing

There were no statistically significant differences in the NMSS total scores between the two groups (p=0.831) as shown in Table 7.4b and Figure 7.13.

Table 7.4b Variation in non-motor symptoms in patients (n=276) classified by family history of PD. (continuation of Table 7.4a)

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	64	212	-
Genitourinary dysfunction			
Bladder dysfunction †	97.0 %	95.3 %	0.584
Erectile dysfunction (males)	56.8 %	57.0 %	0.979
Anorgasmia (females)	40.9 %	45.5 %	0.705
Incontinence (urinary)	30.3 %	37.3 %	0.380
Sleep dysfunction			
RBD **symptoms	37.5 %	41.0 %	0.613
Disrupted sleep‡	40.6 %	47.6 %	0.324
EDS§	37.5 %	34.4 %	0.653
Restless legs symptoms	75.0 %	63.7 %	0.093
Pain(unexplained)	43.8 %	38.2 %	0.427
Fatigue	73.4 %	78.3 %	0.416
NMSS total score	41 (26-70)	39 (23-73)	0.831

FH = family history, IQR = inter-quartile range, † Bladder dysfunction = difficulty retaining urine, feeling bladder not completely empty, weak stream, pis en deux or nocturia. RBD = Rapid eye movement sleep behaviour disorder, **RBD questionnaire score ≥ 5, ‡ using Parkinson's disease sleep scale (PDSS) score > 100, § EDS= excessive daytime sleepiness using Epworth sleep scale (ESS) score ≥ 11, NMSS = non-motor symptom scale.

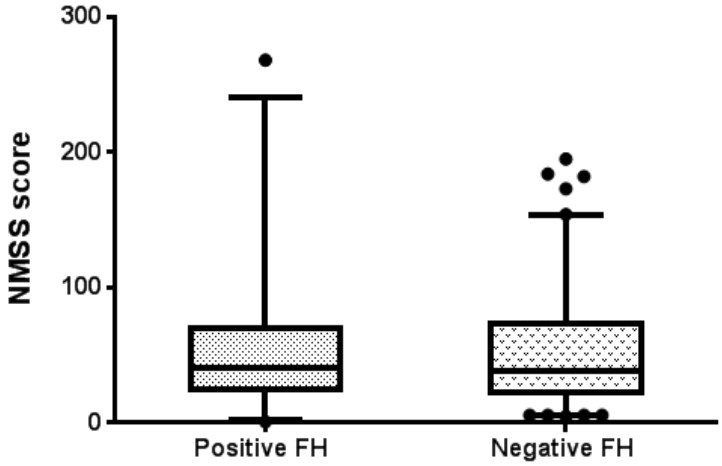

Figure 7.13 shows the non-motor symptoms scale score (on the y-axis) and the family history (FH) of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the NMSS total scores were found between those with familial PD (n=64) compared to those with sporadic PD (n=212) (p=0.831).

7.5 Discussion

Non-motor symptoms of PD are legion and increasingly being recognised by clinicians as a source of considerable distress in the daily lives of these patients. The Parkinson and non-motor symptoms (PRIAMO) study report suggested that the mean number of non-motor symptoms per patient can be 7-8 (range, 0-32) [36]. The PRIAMO study investigators used a semistructured interview to determine the prevalence of NMS. We used the validated NMSS to determine the prevalence of NMS in our cohort as it is free from floor and ceiling effects [358]. The PRIAMO study (n=1072) reported a near universal presence (98.6%) of NMS in their cohort. We found similar results and all of our patients (n=269) had at least one NMS to report. NMSS has nine domains: cardiovascular, sleep/fatigue, mood/cognition, perceptual problems, attention/memory, gastrointestinal, urinary, sexual function and "miscellany" [352]. The "miscellany" domain of the NMSS contains questions about pain, olfaction, weight changes and hyperhidrosis. Based on this scale the prevalence of NMS in each of these domains for the whole cohort (n=269) was: cardiovascular (51%) , sleep/fatigue (67%), mood/cognition (48%), perceptual problems (29%), attention/memory (61%), gastrointestinal (41%), urinary (65%), sexual function (38%) and "miscellany" (49%).

However, some of these domains contain questions that overlap the boundaries of the human body's anatomical and physiological systems. The autonomic nervous system, for example, innervates several organs in the body including the cardiovascular system, skin and bowel. Autonomic system dysfunction can have several manifestations including orthostatic hypotension, anhidrosis and constipation [359]; we have therefore considered questions from the NMSS [352] and SCOPA-Aut [72] related to these organ systems under one domain when classifysing the EOPD cohort according to clinical subtypes (Tables 7.1-7.4). The sleep domain of NMSS has only 4 questions [352] but we considered this barely useful to assess either excessive daytime sleepiness or nocturnal sleep disorders without exploring the potential causes. We used more detailed and validated scales such as the RBD questionnaire (using a cutoff score> 5) [357], Parkinson's disease sleep scale (PDSS) using a cutoff score >100 [360] and Epworth sleep scale (ESS) using a cutoff score >=11[355] to document specific problems in sleep (Tables 7.1-7.4). Sexual and urinary dysfunction are considered as two separate domains in NMSS, however, we considered these symptoms under one domain (genitourinary) as the anatomical and physiological substrates for these symptoms are intimately linked (Tables 7.1-7.4).

In addition to the motor disability of PD non-motor symptoms such as depression, anxiety, fatigue, confusion, autonomic disturbance particularly urinary incontinence and sensory symptoms such as pain were the major predictors of QoL. This has implications for the medical management of PD if QoL is an important therapeutic endpoint [353].

There are reported differences in the prevalence of non- motor symptoms in between the motor subtypes of PD. Some features such as hyper salivation and dribbling are significantly more common in those with the PIGD subtype than in those with TDPD [361]. Our results, however, showed no statistically significant differences in the prevalence of autonomic, gastrointestinal, genitourinary, sleep dysfunction, fatigue or pain symptoms between the three motor subtypes (Tables 7.1a and 7.1b). The previous study reported findings from a cohort of LOPD with different demographic characteristics, hence, is not directly comparable to our study.

Gender based differences in the prevalence of non- motor symptoms has also been reported.

Male patients are reported to more frequently complain of sexual dysfunction and taste/smelling difficulties significantly more frequently than female patients [328]. The prevalence of pain as a symptom in patients with PD ranges from 30 to 70% [362-365] . While some reports have suggested no differences in the prevalence of pain between genders [366], others have reported pain to be more prevalent in female patients [367] . We also found pain to be more commonly reported by females than males in the EOPD cohort ($p=0.022$). The higher prevalence of pain may be related to perceptual differences of pain between genders or lower pain thresholds in females[368]. It is recognised that although pain experienced by patients may have several causes including comorbidities such as osteoarthritis, cases with PD can have pain without an identifiable cause, some clinicians use the terms 'non-PD pain' and 'PD pain' to delineate these two entities [369]. Our study also showed other differences in between the two sexes with more females reporting urinary incontinence ($p<0.001$), prandial bloating ($p=0.024$) and constipation ($p=0.022$) compared to males who had a higher prevalence of dysphagia ($p=0.003$).

The age of onset of PD is reported to influence the development of non-motor symptoms. Old-age at onset is characterized by more olfactory and sensory symptoms, autonomic symptoms and sleep [370]. Our results didn't show any differences in between autonomic or sleep symptoms however sexual dysfunction was more commonly reported by females in the older age group ($p=0.022$).

The prevalence of non-motor symptoms in familial cases of PD compared to sporadic cases has not been reported. We found no difference in the prevalence of non-motor symptoms between the 2 sub-groups except prandial bloating was more commonly reported by those with a family history of PD ($p=0.036$). The significance of this, if any, remains undetermined.

7.6 Conclusion

Non-motor symptoms are common in parkinsonian patients in varying proportions. Unlike motor symptoms the determinants of the heterogeneity in most non- motor symptoms are largely unknown, with exceptions such as cognitive problems (analysed in Chapter 10). Nevertheless the recognition and management of non-motor symptoms is important because of their significant influence on the quality of life of affected patients.

Chapter 8. Variation in olfactory function in EOPD.

8.1 Objective

The primary objective of our work here was to assess olfaction in a cohort of EOPD cases in order to determine the degree or grading of olfactory loss according to motor subtype, age at onset and heritability of the parkinsonian trait. A secondary objective was to analyse the correlation between olfactory, cognitive, motor and non- motor symptom scores.

8.2 Introduction

Olfactory dysfunction is an important and early feature of Parkinson's disease (PD) [371]. The degree of olfactory loss is however variable and is differentially impaired in distinct parkinsonian syndromes [372]. It is also well accepted that PD has a pre-motor phase and olfactory loss is a recognised feature of this phase [373]. Current diagnostic criteria for PD [317] have an emphasis on cardinal motor features, but non-motor symptoms including the loss of sense of smell may precede the motor presentation by several years [50]. Olfactory dysfunction is therefore under study as a biomarker, or screening tool, for premotor PD, in combination with other prodromal features identified through questionnaires and simple motor tasks [374]. This is less invasive and less expensive than other modalities, such as functional dopamine brain scanning.[375] A simple 'scratch and sniff' test such as the University of Pennsylvania smell identification test (UPSIT) has been applied in selected populations e.g. relatives of those affected with PD who have an increased risk of developing PD [374] . There is some evidence that olfactory dysfunction in PD differs according to motor subtype, being more evident in patients with tremor dominant PD (TDPD) than in postural instability gait difficulty motor subtype (PIGD) [376, 377] . These studies had a mix of cases of early onset PD (EOPD) and late onset PD (LOPD) and no study has looked

specifically at EOPD cases. EOPD differs from late onset PD (LOPD) by having slower motor progression, a longer disease course, and greater preservation of cognitive function, but earlier motor fluctuations and dyskinesia [378]. It is also more likely that EOPD cases have a genetic basis to the underlying aetiology compared to LOPD cases when considering genes like *Parkin.* [169]

8.3 Methods

Early onset PD cases (age <50 years at diagnosis) based on UK Brain Bank criteria [317], enrolled in the PRoBaND study were included. Patients with features indicating other types of degenerative parkinsonism e.g. progressive supranuclear palsy, drug-induced or vascular parkinsonism, and those with normal presynaptic dopamine imaging (performed in selected cases on clinical grounds) were excluded. All patients had demographic data, relevant past medical history, vital signs, diagnostic features at presentation, medication history and family history recorded. Smell testing was performed using the British version of the UPSIT, a 40-odour 'scratch and sniff' test.

Classification of cases: All cases were classified into TDPD, PIGD and 'Mixed', based on UPDRS part 2 (history) and part 3 (examination) scores, was done as explained in detail in Chapter 4. Further exploratory analysis involved classifying cases by age of onset at PD and family history of PD (hereditary vs. sporadic PD). In all classes cases were grouped by gender as differences in olfactory acuity based on gender are well recognised.

Definitions of normosmia and olfactory dysfunction: Standard definitions were applied to grade olfactory performance as follows: anosmia - UPSIT scores<19 for both males and females); hyposmia- UPSIT scores 19-33 inclusive in men, and 19-34 inclusive in women; and normosmia - UPSIT scores >34 for men and >35 for women. Hyposmia was sub-categorized as mild - UPSIT scores of 30-33 inclusive in men, and 31-34 inclusive in women); moderate - UPSIT scores of 26-29 inclusive in men, and 26-30 inclusive in women), and severe (UPSIT scores of 19-25, inclusive, in men and women).[379]

Statistical analyses: The UPSIT scores (dependent variable) were compared using a generalised linear model, based on ANCOVA, using age and disease duration as covariates and motor subtype and gender as fixed factors (independent variables) . Logarithmic transformations (log10) were obtained prior to fitting the data in the statistical model where it failed a test of normality (Shapiro Wilk). Chi-square tests were used for categorical variables. Those with age at diagnosis ≤ 40 years and those with age at diagnosis > 40 years were also compared using a similar generalised linear model described above and Fisher's exact test for categorical data. Associations among the different measures were tested using correlation analyses (Pearson for parametric, Spearman for non-parametric). All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using MINITAB software (*version 16 for Windows*; PA, USA) and SPSS software (*version 23 for Windows*; IL, USA).

8.4 Results

213 patients had filled in all the booklets from the UPSIT, relevant to this analysis and are included here (Figure 8.1).

There were 136 males (63.6 %) and 77 females (34.4%), median age 52.1 years (interquartile range, IQR, 47.5-56.6 years), median disease duration 7.3 years (IQR 3.0-11.4 years). The median UPSIT score was 21 out of 40 (IQR 16-28) for the entire cohort. Females scored higher (median 27, IQR 20-30) than males (median 19, IQR 14-25, $p<0.0001$). (Figure 8.2)

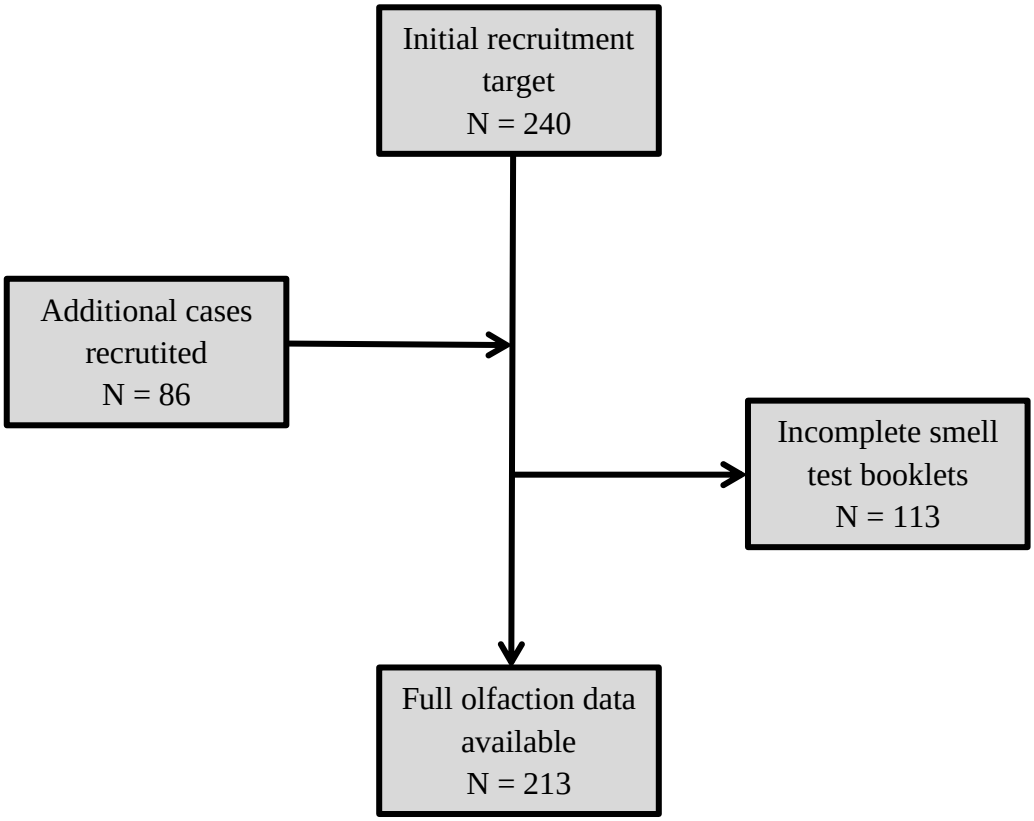

Figure 8.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

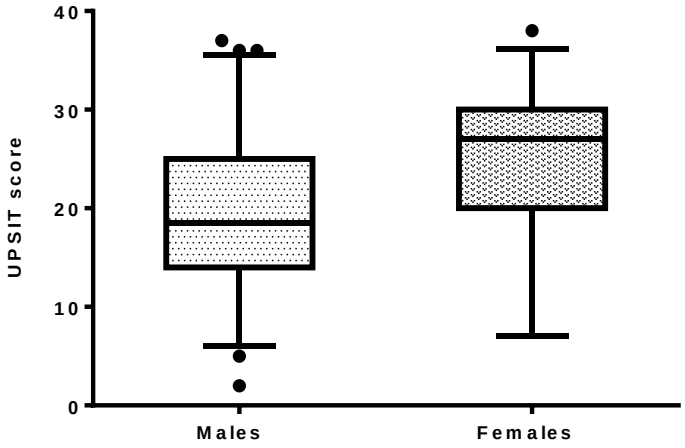

Figure 8.2 shows box-whisker plots of the scores on the University of Pennsylvania smell identification test 40 item version (UPSIT) on the y-axis versus gender on the x-axis. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. Females obtained higher scores compared to males ($p<0.0001$).

There were no statistically significant differences in the UPSIT scores between the TDPD, PIGD and 'Mixed' motor subtypes ($p=0.523$) (Figures 8.3 and 8.4).

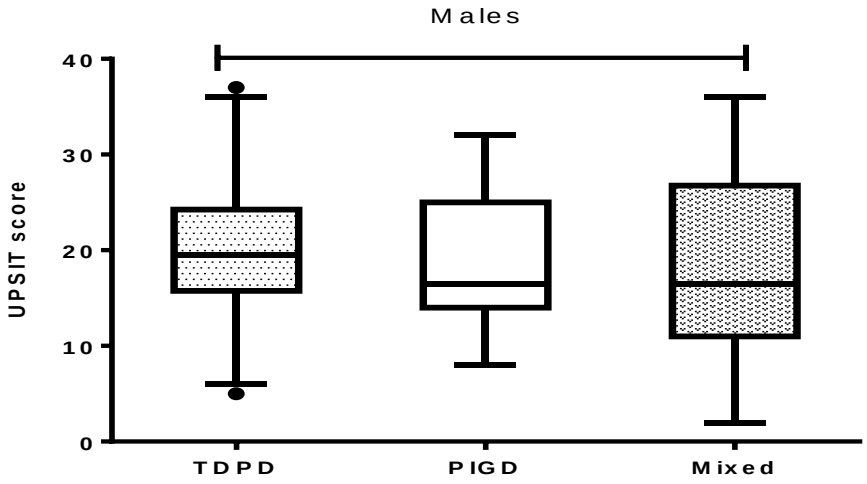

Figure 8.3 shows box-whisker plots of the scores on the University of Pennsylvania smell identification test 40 item version (UPSIT) on the y-axis versus motor subtype of males (n=136) on the x-axis. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the UPSIT scores were found between the three motor subtypes amongst males (p=0.454).

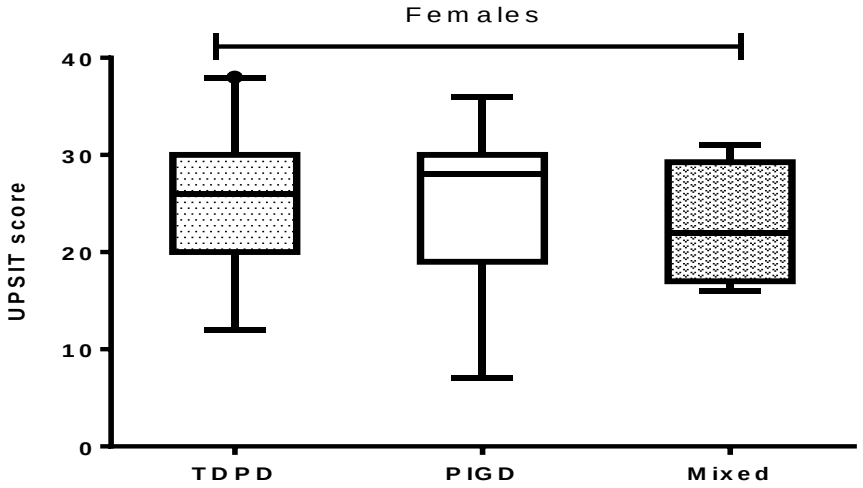

Figure 8.4 shows box-whisker plots of the scores on the University of Pennsylvania smell identification test 40 item version (UPSIT) on the y-axis versus motor subtype in females on the x-axis. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences in the UPSIT scores were found between the three motor subtypes amongst females (p=0.765).

The proportion of patients with normosmia, 3 grades of hyposmia and anosmia classified by motor subtype is shown in Table 8.1.

Table 8.1 Olfactory status in patients (n=211) classified by motor subtype and gender.

Olfactory status*	**TDPD** median (IQR)	**PIGD** median (IQR)	**'Mixed'** median (IQR)
Number of cases	117	79	15
Age***** (in years)	50.9 (46.0-54.3)	54.9 (49.8-59.8)	46.7 (41.5-51.0)
Sex (males)	66.1 %	59.3 %	68.8 %
Duration (years)	5.5 (2.4-9.8)	9.1 (4.9-15.0)	7.5 (2.4-11.0)
UPSIT score	M: 20 (16-24) F: 26 (20-30)	M: 17 (14-25) F: 28 (20-30)	M: 17 (11-22.2) F: 24 (22-28)
Normosmia	M: 5.1% F: 12.5%	M: 0 % F: 6.1 %	M: 8.3 % F: 0 %
Mild hyposmia	M: 5.1 % F: 17.5 %	M: 10.9 % F: 24.2 %	M: 0 % F: 33.3 %
Moderate hyposmia	M: 11.5 % F: 22.5 %	M: 8.7 % F: 30.3 %	M: 16.7 % F: 0 %
Severe hyposmia	M: 32.1 % F: 32.5 %	M: 26.1 % F: 15.2 %	M: 16.7 % F: 66.7 %
Anosmia	M: 46.2 % F: 15.0 %	M: 54.3 % F: 24.2 %	M: 58.3 % F: 0 %
Total (hyposmia or anosmia)	M: 94.9 % F: 87.5 %	M: 100 % F: 93.9 %	M: 91.7 % F: 100 %

TDPD = Tremor dominant Parkinson's disease, PIGD-PD = Postural instability gait difficulty dominant Parkinson's disease, *using gender based cut-off scores from the University of Pennsylvania Smell Identification 40 item test, M = male, F = female.

There were no statistically significant differences in the UPSIT scores between those with age at onset ≤ 40 years compared to those with age at onset > 40 years (p=0.243). The proportion of patients with normosmia, 3 grades of hyposmia and anosmia classified by age at diagnosis of PD in Table 8.2 and in Figures 8.5 and 8.6.

Subgroup analysis showed males with a relatively earlier onset of disease (≤ 40 years of age) had lower UPSIT total scores (median 22, IQR 16-28) compared to females (median 28, IQR 20-30) (p= 0.0179). This trend persisted in those with later onset of disease (> 40 years of age) with females achieving higher scores on UPSIT (median 26, IQR 20-30) compared to males (median 19, IQR 14-24) (p<0.001)

Table 8.2 Olfactory status in patients (n=211) classified by age at onset of PD and gender.

Olfactory status*	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset >40 years** median (IQR)
Number of cases	45	166
Age† (in years)	46.7 (41.5-51.0)	53.1 (49.4-57.2)
Sex (males)	69.6 %	62.1 %
Disease duration (years)	8.2 (3.6-15.1)	6.9 (2.8-11.2)
UPSIT scores	M: 21 (16-28) F: 28 (20-30)	M: 18 (14-24) F: 26 (20-30)
Normosmia	M: 0 % F: 15.4 %	M: 4.9 % F: 7.9 %
Mild hyposmia	M: 12.1 % F: 15.4 %	M: 4.9 % F: 22.2 %
Moderate hyposmia	M: 18.2 % F: 30.8 %	M: 8.7 % F: 23.8 %
Severe hyposmia	M: 21.2 % F: 30.8 %	M: 31.1 % F: 25.4 %
Anosmia	M: 48.5 % F: 7.7 %	M: 50.5 % F: 20.6 %
Total (hyposmia or anosmia)	M: 100 % F: 84.6 %	M: 95.1 % F: 92.2 %

*** using gender based cut-off scores provided in the University of Pennsylvania Smell Identification test kit (40 item) British version. † age at registration, M = male, F = female.**

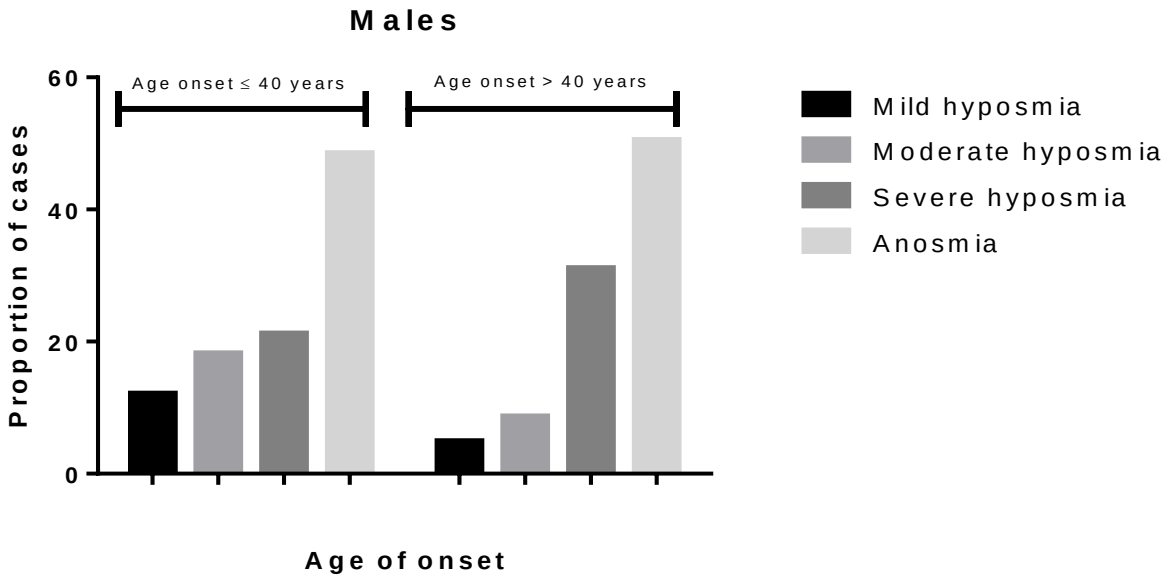

Figure 8.5 shows the proportion of male patients with varying grades of hyposmia and anosmia in those with age of diagnosis ≤ 40 years (n=64), in the left 4 bars, and in those with age of diagnosis > 40 years (n=214), in the right 4 bars in the graph. [Note: Normosmic cases are not shown.]

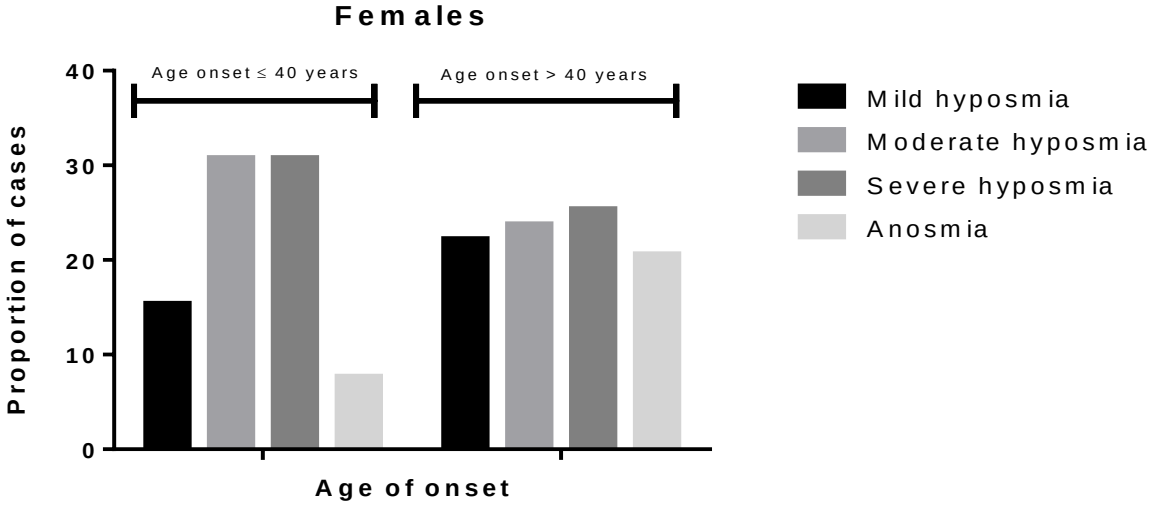

Figure 8.6 shows the proportion of female patients with varying grades of hyposmia and anosmia in those with age of diagnosis ≤ 40 years (n=64), in the left 4 bars, and in those with age of diagnosis > 40 years (n=214), in the right 4 bars in the graph. [Note: Normosmic cases are not shown.]

There were no statistically significant differences in the UPSIT scores between those with a family history of PD compared to those without a family history of PD (p=0.385). The proportion of patients with normosmia, 3 grades of hyposmia and anosmia and classified by family history of PD in Table 8.3.

Table 8.3 Olfactory status in patients (n=211) classified by family history of PD and gender.

Olfactory status*	**Positive FH** median (IQR)	**Negative FH** median (IQR)
Number of cases	52	159
Age***** (in years)	52.1 (47.4-56.8)	52.0 (47.5-56.6)
Sex (males)	63.5 %	63.8 %
Disease duration (years)	6.4 (3.3-11.5)	7.4 (2.8-11.3)
UPSIT scores	M: 15 (20-25) F: 21 (28-31)	M: 14 (18-25) F: 20 (26-30)
Normosmia	M: 0 % F: 10.5 %	M: 4.9 % F: 8.6 %
Mild hyposmia	M: 8.8 % F: 26.3 %	M: 5.9 % F: 19.0 %
Moderate hyposmia	M: 14.7 % F: 26.3 %	M: 9.8 % F: 24.1 %
Severe hyposmia	M: 29.4 % F: 21.1 %	M; 28.4 % F: 27.6 %
Anosmia	M: 47.1 % F: 15.8 %	M: 51.0 % F: 20.7 %
Total (hyposmia or anosmia)	M: 100 % F: 89.5 %	M: 95.1 % F: 91.4 %

FH = family history, * using gender based cut-off scores provided in the University of Pennsylvania Smell Identification test kit (40 item) British version. M = male, F = female.

Subgroup analysis showed males with a positive family history of PD had lower UPSIT total scores (median 20, IQR 15-25) compared to females (median 28, IQR 21-31) (p= 0.021). This trend persisted in those with negative family history of PD i.e. sporadic PD with females achieving higher scores on UPSIT (median 26, IQR 20-30) compared to males (median 18, IQR 14-25) (p=0.001).

There was a weak but significant positive correlation between UPSIT and MOCA test score (Spearman's rho=0.177, p<0.001) and weak negative correlations between UPSIT scores and UPDRS3 (Spearman's rho= -0.0272, p=<0.001), NMSS (Spearman's rho= -0.177, p=0.010) and PDQ8SI (Spearman's rho= -0.153, p=0.027).

Discussion

EOPD cases are an important subset to identify for studies of neuroprotection, given their longer disease duration and a tool to detect pre-morbid PD would therefore be useful. Olfactory loss on its own may not be a perfect tool for identifying pre-motor PD because of its low specificity however this can be increased by using smell tests in a 2 step combined approach where the presence of olfactory loss in combination with other non-motor features such REM sleep behaviour disorder could be a pointer towards pre-motor PD [380], thereby helping select at risk patients who could then be assessed for evidence of pre-synaptic neurodegeneration using dopamine transporter imaging. This approach has been used in population based studies such as the Parkinson At-Risk Syndrome (PARS) study [172]. Besides its diagnostic value as a test olfactory dysfunction can have prognostic value too as odour identification deficits are associated with increased risk of neuropsychiatric complications and cognitive decline in patients with Parkinson's disease [381]. Therefore the utility of olfactory testing in PD is twofold i.e. for diagnostic and perhaps prognostic purposes with the added double bonus advantage of it being 'low tech' and 'low cost' compared to all other screening modalities with a similar sensitivity. However if olfactory dysfunction is to be used as screening tool for premotor PD, it is important that this should not select cases with a particular subtype of PD at the expense of other subtypes.

The prevalence of olfactory dysfunction in PD in general is about 90-95% [382] but there are significant variations with lower rates in some genetic variants of PD such as those with *Parkin* and *DJ1* mutations [383, 384]. While these monogenic forms of PD account for a small number of total PD cases, they are more likely to be present in EOPD and therefore affect olfactory scores. Using arbitrary single point cut-offs classifies patients into either normosmic and hyposmic or normosmic and anosmic, depending upon which cut-off is used, but the fact is olfactory loss varies by degrees in the population and exists on a spectrum from mild, moderate and severe hyposmia through to anosmia (and functional olfactory loss) influenced by age and sex [379, 385]. We therefore classified patients in all clinical subtypes by grades of olfactory loss and gender. The British version of the UPSIT was used as the standard version (North American) contains some smells such as root beer, and words such as gasoline, that are not familiar in the British population.

There are significant differences between EOPD and LOPD in motor, non-motor, cognitive and quality of life domains [378]. Previous reports indicate that there are significant differences in olfactory acuity in the 2 major motor subtypes of PD i.e. TDPD have higher UPSIT scores than PIGD in both males and females but these reports have included a mix of EOPD and LOPD[376, 377] cases or have not clearly demarcated cases with a 'Mixed' motor subtype [376] or failed to demarcate by age of onset [377]. Our analysis has taken account of these inconsistencies and we have appropriately analysed data by age at diagnosis, used a more homogeneous population with only EOPD cases and motor sub-typed all patients without ignoring those classified as 'Mixed'. Prior studies by classifying PD into motor subtypes have also shown significant differences in the rate of cognitive decline [386], motor impulsivity [387], psychopathological features (anxiety and depression) [388], functional disability [179] and even mortality [389] providing evidence that these are not artificially created categories but different clinical subtypes with variations in motor, non-motor and cognitive domains. A correlation of olfactory loss with poor cognitive performance has been demonstrated in LOPD cases [390], our data showed similar results with a weak but statistically significant correlation between UPSIT and MOCA test scores.

We confirmed that patients with EOPD also have differences in olfactory scores based on gender as has been reported for patients with late LOPD but we failed to find any significant differences in the UPSIT scores in patients based on motor subtypes, age at onset of PD

(within the EOPD cohort) and hereditary versus sporadic PD. Females scored better than males in all clinical subtypes examined in this study, clearly demonstrating gender differences in this variable in all subgroups.

There are some limitations to our study. Although head trauma can cause olfactory dysfunction we did not exclude cases who gave a past history of head trauma without specifying that it showed a temporal relationship to their subjective loss of sense of smell. Our study was also limited by the fact that no anterior rhinoscopy was performed to specifically look out for local causes of airflow obstruction in the nose. We looked at cross-sectional data for olfaction in the EOPD cohort and don't have data about olfaction early in the disease course noting that earlier research indicates no relation of olfaction to disease duration[63] although some researchers have drawn opposite conclusions[66].

In using the more recently available British version of the UPSIT, our results are less directly comparable to other studies in the United Kingdom [374] which have used the traditional North American version, but are more readily comparable to North American studies of olfaction in PD, or other country or region-specific versions of UPSIT. We consider the use of normative data based on the North American version to be reasonable, in the absence of similar data for the United Kingdom, as there is significant overlap between the smells used (30 of 40 odours are common to the 2 versions).

Conclusion

In early onset PD, motor subtyping, age at onset and hereditary versus sporadic nature of PD do not show significant differences in olfactory sores on the University of Pennsylvania smell identification test. More detailed studies are required to establish if there is a link in the pathology underlying the olfactory and cognitive dysfunction given the correlation in scores on tests recording these variables.

Chapter 9. Variation in cognitive problems in EOPD

9.1 Objective

The objective in this chapter is to analyse the variation in the cognitive problems in a cohort of patients with early onset Parkinson's disease.

9.2 Introduction

Cognitive problems are common in Parkinson's disease (PD) patients, the impairments caused affect quality of life and the ensuing problems with activities of daily living add significantly to the burden of disease. These problems exist on a spectrum from mild cognitive impairment to PD dementia (PDD). Longitudinal studies such as the Sydney multicentre study suggest that up to 80% of patients with PD eventually develop dementia [115]. PDD is treatable with cognition enhancers (cholinesterase inhibitors) and research in this field is an on-going enterprise in an effort to develop more specific therapies for PD-related cognitive problems.

9.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0). Patients were asked about their subjective feelings of impaired memory and concentration at visit 1 as part of their NMSS questionnaire. This was then compared with the results of objective testing with the Montreal cognitive assessment (MOCA) test at the same visit. Details of schooling or education for collation to MOCA test scores was obtained at visit 3 as part of a social history questionnaire.

Normal cognition based on MOCA test score was defined as greater than or equal to 26, cognitively impaired as defined as a score less than 26 which was further sub classified into mild cognitive impairment (MCI) with a score less than 26 [391] but greater than or equal to 20 and dementia with a score of less than 20 [392].

In order to analyse for the variation in non-motor phenotype of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed'), gender, age at onset of PD symptoms and family history of Parkinson's disease as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

Generalised linear modelling, based on ANCOVA, was used with age ,disease duration and years of schooling as covariates to determine the effect of the motor subtype, gender, age at onset and hereditary versus sporadic parkinsonism (independent variables) on the MOCA test scores (dependent variable). Logarithmic transformations (log 10) of numerical data were used where tests of normality (Shapiro Wilk) failed.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (version *23 for Windows*; IL, USA).

9.4 Results

276 patients had completed all questionnaires relevant to this analysis and are included here (Figure 9.1). Their demographic details have been presented in Table 5.1 in Chapter 5.

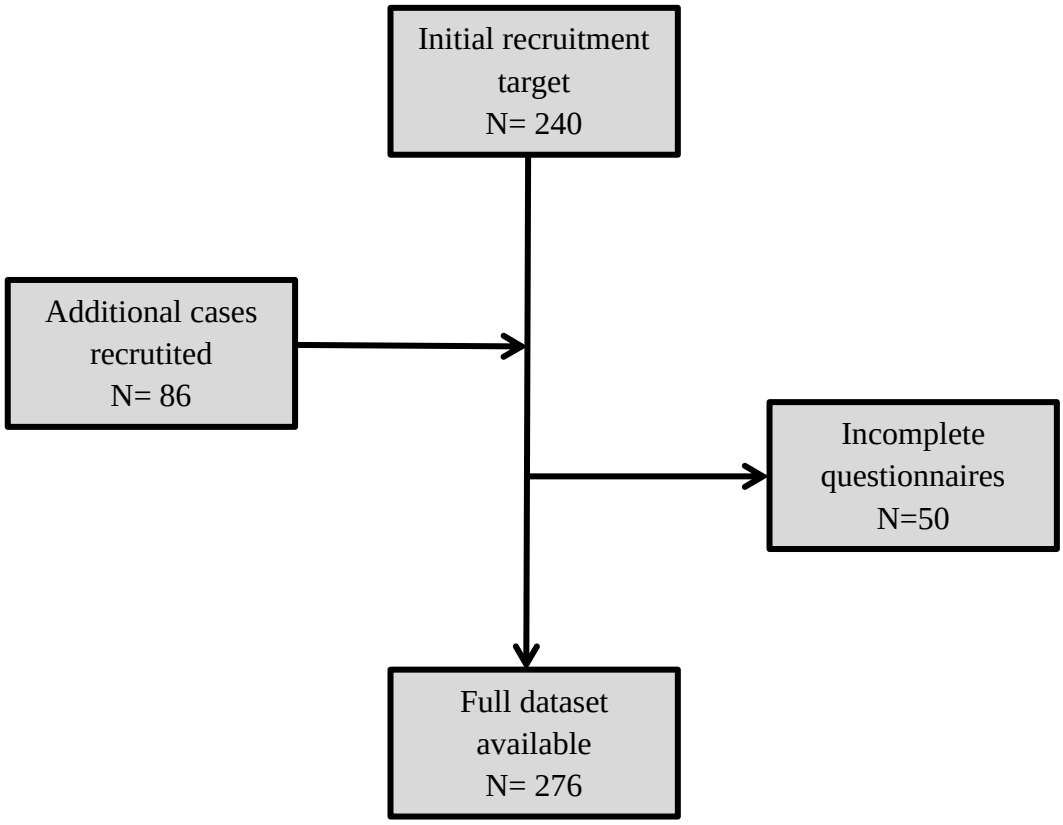

Figure 9.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in the prevalence of cognitive problems in these patients, classified by motor subtype, is presented in Tables 9.1a and 9.1b. There were no statistically significant differences in the median MOCA test total scores (Figure 9.2) between the three motor subtypes (Table 9.1a) but using cut-offs to define “normal”, mild cognitive impairment and dementia, as defined in the methods section, showed significant differences between the 3 motor subtypes (Figure 9.3).

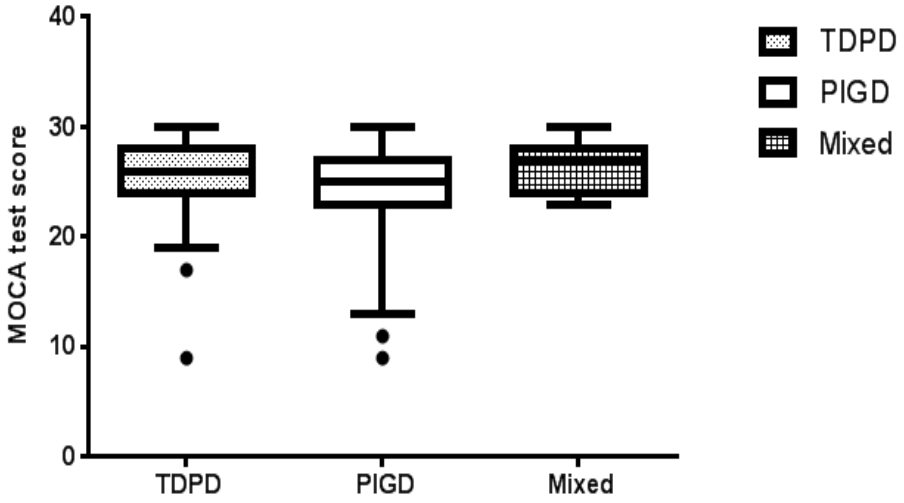

Figure 9.2 shows the Montreal cognitive assessment (MOCA) test score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found in the the MOCA test scores between those with TDPD (n=155), PIGD (n=100) or 'Mixed' (n=21) motor subtypes (p=0.183) after adjusting for age, disease duration and years of schooling.

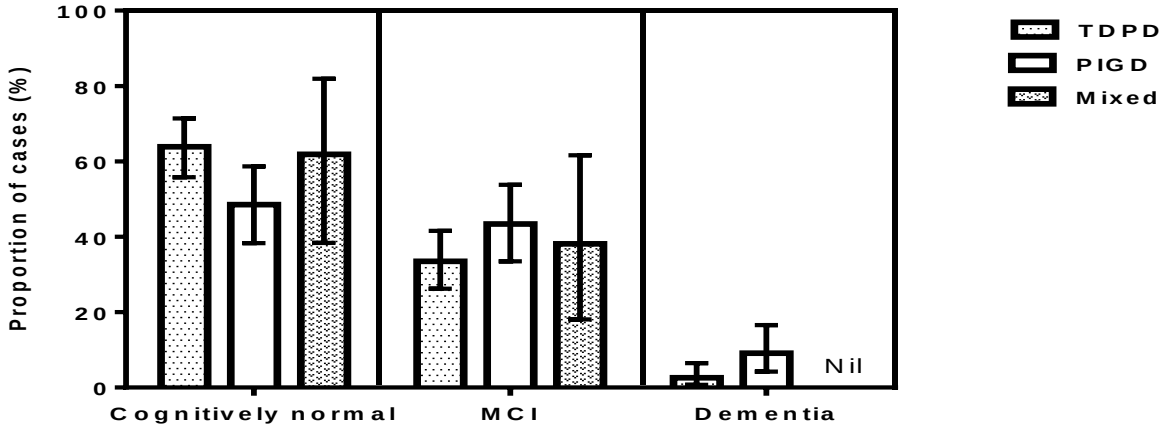

Figure 9.3 shows the proportion of patients who were cognitively normal, had mild cognitive impairment (MCI) or dementia (on the y-axis) as recorded on the MOCA test with respect to their motor subtype.

Post hoc tests showed more PIGD patients to be affected with cognitive impairment compared to TDPD (p=0.015) but not between TDPD and 'Mixed' (p=1.000) or between PIGD and 'Mixed' (p=0.339). Dementia was also more prevalent in the PIGD group compared to the TDPD group (p=0.013). No patients in the 'Mixed' subgroup were classified as demented (Table 9.1a).

Table 9.1a Variation in cognitive profiles in patients (n=276) classified by motor subtype.

Variable	**TDPD** mean (SD)	**PIGD** mean (SD)	**Mixed** mean (SD)	**p-value**
Number of cases	155	100	21	-
Age (at registration, in years)	49.8 (6.7)	54.4 (9.0)	50.5 (5.8)	**<0.001**
Sex (males)	67.7 %	59.0 %	65.0 %	0.356
Disease duration (years)	6.2 (5.2)	10.8 (9.3)	7.8 (6.7)	**<0.001**
Age at leaving school (years)	16.6 (1.0)	16.2 (1.0)	16.6 (1.1)	**0.030**
Impaired concentration (s)	65.6 %	73.7 %	70.0 %	0.391
Impaired memory (s)	75.5 %	82.8 %	80.0 %	0.380
MOCA test score	25.9 (2.9)	24.4 (4.5)	26.4 (2.3)	0.183
Normal (≥ 26)	63.9 %	48.5 %	61.9 %	-
Cognitive impairment (< 26)	36.1 %	51.5 %	38.1 %	**0.049**
MCI PD (≥ 20, < 26)	33.5 %	43.4 %	38.1 %	0.140
Dementia (< 20)	2.6 %	9.1 %	0	**0.013**

SD= standard deviation. MCI PD= Mild cognitive impairment Parkinson's disease. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher. (s) = self-reported

Detailed analysis of the MOCA test scores showed significant differences between the 3 motor subtypes in the domains of language [sub-domains naming (p=0.001), category fluency (p=0.031)], registration (p=0.020), short term memory as represented by delayed recall (p=0.029) but not working memory as represented by the forward and backward digit span (p=0.522). There were also statistically significant differences between the 3 sub-groups

in the domains of executive function [subdomain abstraction (p=0.022)] and visuospatial abilities as represented by copying a cube (p=0.042).

Table 9.1b is a continuation of Table 9.1a and shows detailed analysis of MOCA test scores of patients (n=276) classified by motor subtype.

Variable	**TDPD** mean (SD)	**PIGD** mean (SD)	**Mixed** mean (SD)	**p-value**
Number of cases	155	100	21	-
MOCA test scores				
Trail	0.89 (0.31)	0.83 (0.38)	0.81 (0.40)	0.302
Cube	0.87 (0.34)	0.75 (0.44)	0.86 (0.36)	**0.042**
Clock	3.00 (1.00)	2.59 (0.73)	2.86 (0.36)	0.063
Pentagons*	0.93 (0.26)	0.89 (0.31)	1.00 (0.00)	0.199
Naming	2.99 (0.11)	2.87 (0.42)	3.00 (0.00)	**0.001**
Registration	4.75 (0.68)	4.40 (1.13)	4.71 (0.72)	**0.020**
Memory†	3.32 (1.39)	2.72 (1.78)	3.52 (1.36)	**0.029**
Digit span‡	1.86 (0.34)	1.84 (0.44)	1.95 (0.22)	0.522
Vigilance	0.90 (0.30)	0.89 (0.31)	0.99 (0.30)	0.939
Serial 7's (attention)	2.37 (0.76)	2.22 (0.77)	2.48 (0.75)	0.116
Repetition	1.81 (0.47)	1.70 (0.59)	1.71 (0.64)	0.290
Lexical fluency	0.66 (0.47)	0.74 (0.44)	0.71 (0.46)	0.433
Category fluency* §	23.97 (7.25)	21.44 (6.82)	23.1 (6.64)	**0.031**
Abstraction	1.75 (0.54)	1.53 (0.73)	1.81 (0.51)	**0.022**
Orientation	5.75 (0.77)	5.72 (0.79)	5.81 (0.40)	0.930

SD = standard deviation. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher. * not part of Montreal cognitive assessment test, † delayed recall, ‡ working memory, §over 90 seconds

Post hoc analysis showed significant differences in naming between TDPD and PIGD ($p<0.001$) but not between PIGD and 'Mixed' ($p=0.209$) or TDPD and 'Mixed' ($p=0.999$). Similar results were obtained on post hoc tests for registration and delayed recall with significant differences between TDPD and PIGD ($p=0.005$, $p=0.015$) but not between PIGD and 'Mixed' ($p=0.239$, $p=0.072$) or TDPD and 'Mixed' ($p=0.892$, $p=0.535$).

There were no differences in the median MOCA test total scores (Figure 9.4) and the proportion of patients with normal or impaired cognition, including dementia, between males and females (Figure 9.5, Table 9.2a)

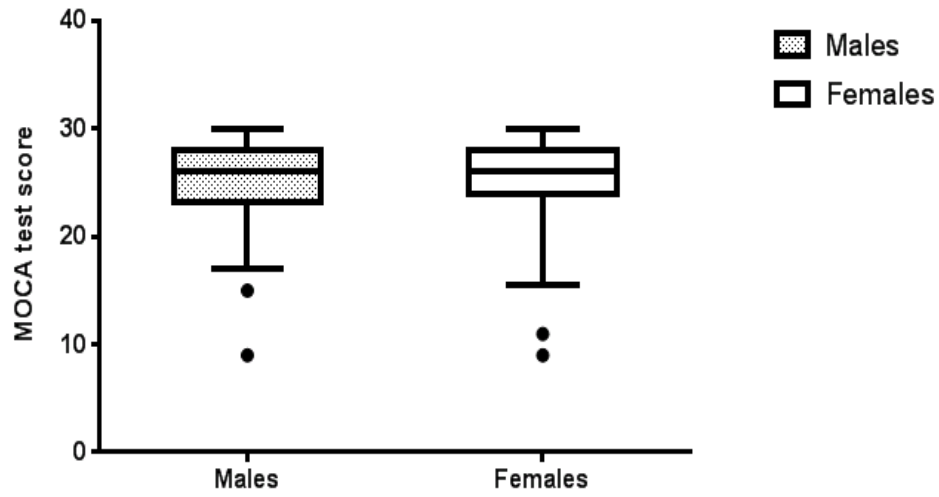

Figure 9.4 shows the Montreal cognitive assessment (MOCA) test score (on the y-axis) and the gender (on the x-axis) of the patients (n=276) in the EOPD cohort of the PRoBaND study. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found in the MOCA test scores between males (n=177) and females (n=99) after adjusting for age, disease duration and years of schooling (p=0.331).

Table 9.2a Variation in cognitive profiles in patients (n=276) classified by gender.

Variable	**Males** mean (SD)	**Females** mean (SD)	p-value
Number of cases	177	99	-
Age* (years)	51.6 (7.9)	51.4 (7.8)	0.968
Age at leaving school (years)	16.5 (1.0)	16.5 (1.1)	0.737
Disease duration (years)	7.9 (6.3)	8.1(7.3)	0.922
Impaired concentration (s)	68.9 %	57.6 %	0.058
Impaired memory (s)	76.3 %	70.7 %	0.311
MOCA test score	25.4 (3.3)	25.8 (3.4)	0.331
Normal (≥ 26)	56.3 %	61.6 %	0.386
Cognitive impairment (< 26)	43.7 %	38.4 %	0.386
MCI PD (≥ 20, < 26)	38.1 %	36.4 %	0.602
Dementia (< 20)	5.7 %	2.0 %	0.214

*** Age at registration, SD = standard deviation. MCI PD = Mild cognitive impairment Parkinson's disease. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher, (s) = self-reported**

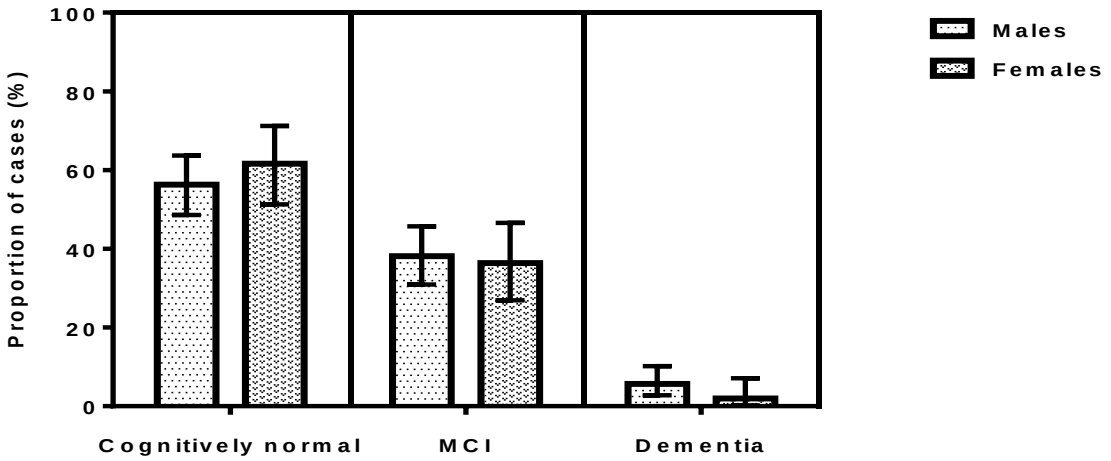

Figure 9.5 shows the proportion of patients who were cognitively normal, had mild cognitive impairment (MCI) or dementia (on the y-axis) as recorded on the MOCA test with respect to their gender.

Detailed analysis of the MOCA test scores showed significant differences between the 2 genders only in the visuospatial domain (p=0.042) as represented by asking the patients to copy a cube (Table 9.2b).

Table 9.2b is a continuation of Table 9.2a and shows detailed analysis of MOCA test scores of patients (n=276) classified by gender.

Variable	**Males** mean (SD)	**Females** mean (SD)	p-value
Number of cases	177	99	-
MOCA test scores			
Trail	0.86 (0.35)	0.88 (0.33)	0.715
Cube	0.86 (0.34)	0.77 (0.42)	**0.042**
Clock	2.72 (0.57)	2.73 (0.62)	0.582
Pentagons*	0.92 (0.27)	0.93 (0.26)	0.791
Naming	2.95 (0.22)	2.97 (0.17)	0.547
Registration	4.57 (0.94)	4.76 (0.61)	0.116
Memory†	3.01 (1.59)	3.35 (1.48)	0.081
Digit span‡	1.90 (0.30)	1.82 (0.44)	0.139
Vigilance	0.90 (0.30)	0.91 (0.29)	0.835
Serial 7's (attention)	2.35 (0.74)	2.31 (0.78)	0.802
Repetition	1.74 (0.53)	1.83 (0.50)	0.069
Lexical fluency	0.69 (0.46)	0.72 (0.45)	0.607
Category fluency* §	23.17 (7.19)	22.90 (6.69)	0.653
Abstraction	1.65 (0.65)	1.73 (0.55)	0.512
Orientation	5.76 (0.73)	5.77 (0.55)	0.628

SD = standard deviation. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher, * not part of Montreal cognitive assessment test, †delayed recall, ‡ working memory, §over 90 seconds

Classifying patients by age at onset of PD into those who had onset of PD age less than (or equal to) 40 years and those who had onset of PD after the age of 40 years also showed no differences in the median MOCA test total scores (Figure 9.6) and the proportion of patients with normal or impaired cognition, including dementia, between males and females (Figure 9.7, Table 9.3a)

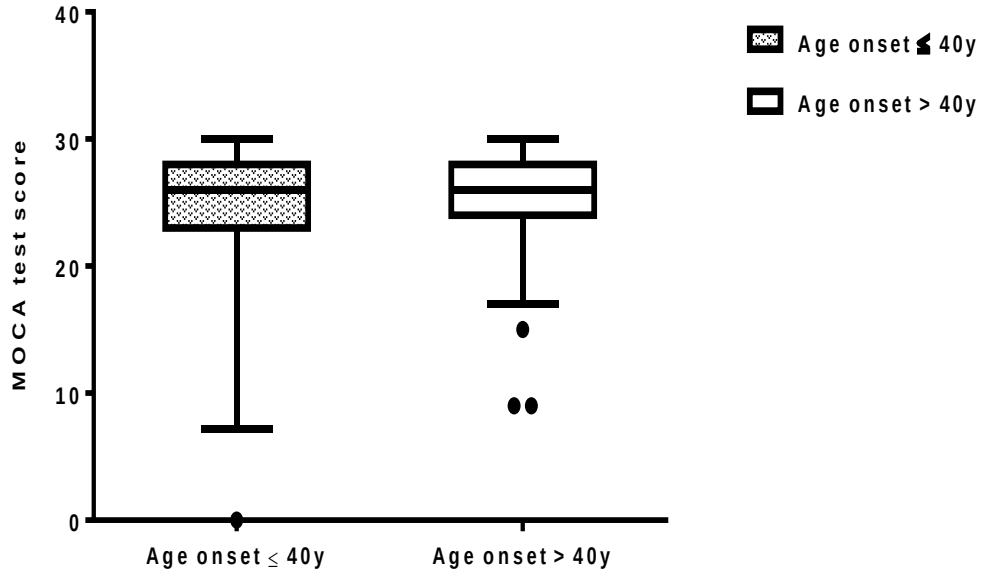

Figure 9.6 shows the Montreal Cognitive Assessment (MOCA) test score of the cases (on the y-axis) and age at onset of PD symptoms (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found in the MOCA test scores between those with age at onset of PD ≤ 40 years (n=64) and those with age at onset of PD > 40 years (n=212) after adjusting for age, disease duration and years of schooling (p=0.966).

Table 9.3a Variation in cognitive profiles in patients (n=276) classified by age at onset of PD.

Variable	**Age onset ≤ 40y** mean (SD)	**Age onset > 40y** mean (SD)	p-value
Number of cases	64	212	-
Age* (years)	45.8 (9.4)	53.3 (6.4)	<0.001
Sex (males)	67.2 %	63.2 %	0.561
Age at leaving school (years)	16.4 (1.1)	16.5 (1.1)	0.354
Disease duration (years)	7.9 (8.6)	6.6 (5.8)	0.707
Impaired concentration (s)	77.8 %	66.5 %	0.082
Impaired memory (s)	81.0 %	77.5 %	0.591
MOCA test score	25.03 (4.47)	25.53 (3.36)	0.966
Normal (≥ 26)	54.7 %	59.2 %	0.517
Cognitive impairment (< 26)	45.3 %	40.8 %	0.517
MCI PD (≥ 20, < 26)	43.8 %	35.5 %	0.325
Dementia (< 20)	3.1 %	5.2 %	0.737

*** Age at registration, SD = standard deviation. MCI PD = Mild cognitive impairment Parkinson's disease. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher, (s) = self-reported**

Detailed analysis of the MOCA test scores showed no significant differences between the 2 subgroups classified by age at onset of PD (Table 9.2b).

Table 9.3b is a continuation of Table 9.3a and shows detailed analysis of MOCA test scores of patients (n=276) classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** mean (SD)	**Age onset > 40 yrs.** mean (SD)	p-value
Number of cases	64	212	-
MOCA test scores			
Trail	0.88 (0.33)	0.86 (0.35)	0.838
Cube	0.82 (0.39)	0.83 (0.38)	0.852
Clock	2.68 (0.71)	2.71 (0.61)	0.954
Pentagons*	0.89 (0.31)	0.93 (0.26)	0.431
Naming	2.91 (0.42)	2.94 (0.29)	0.600
Registration	4.57 (0.95)	4.61 (0.92)	0.463
Memory†	3.29 (1.47)	3.04 (1.61)	0.412
Digit span‡	1.78 (0.48)	1.87 (0.36)	0.100
Vigilance	0.83 (0.38)	0.92 (0.28)	0.057
Serial 7's	2.34 (0.67)	2.31 (0.80)	0.759
Repetition	1.74 (0.59)	1.76 (0.53)	0.894
Lexical fluency	0.62 (0.49)	0.71 (0.45)	0.124
Category fluency* §	22.82 (7.45)	23.00 (7.26)	0.917
Abstraction	1.62 (0.65)	1.68 (0.62)	0.324
Orientation	5.54 1.17)	5.78 (0.68)	0.070

SD = standard deviation. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher, * not part of Montreal cognitive assessment test, †delayed recall, ‡ working memory, §over 90 seconds

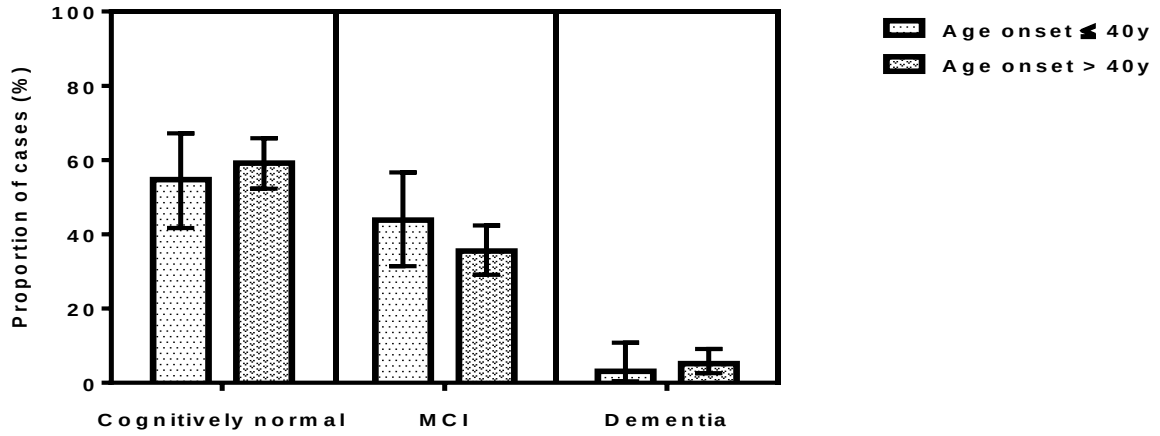

Figure 9.7 shows the proportion of patients who were cognitively normal, had mild cognitive impairment (MCI) or dementia (on the y-axis) as recorded on the MOCA test with respect to their age at onset of PD.

There were no statistically significant differences in the MOCA test scores between those with and without a positive family history of PD (Figure 9.8) and the proportion of patients with normal or impaired cognition, including dementia, between the 2 sub-groups (Figure 9.9, Table 9.4a).

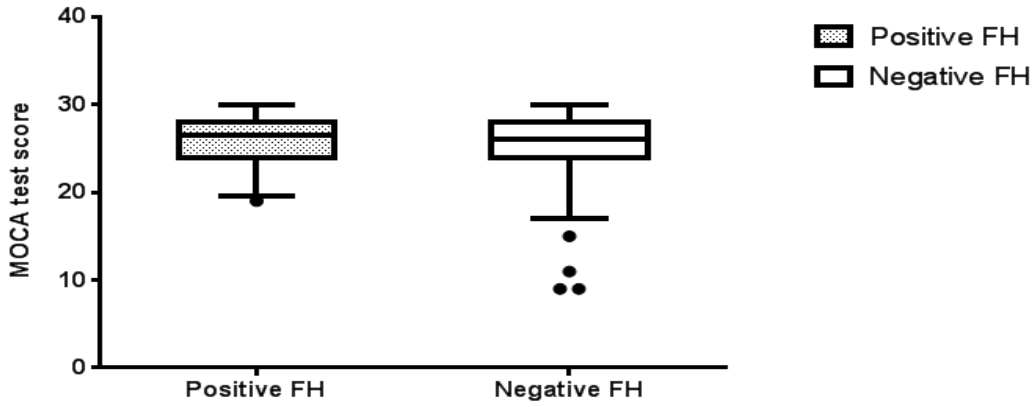

Figure 9.8 shows the Montreal Cognitive Assessment (MOCA) test score of the cases (on the y-axis) and the family history of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found in the MOCA test scores between those with familial PD (n=64) and those with sporadic PD (n=212) after adjusting for age, disease duration and years of schooling (p=0.486).

Table 9.4a Variation in cognitive profiles in patients (n=276) classified by family history of PD.

Variable	**Positive FH** mean (SD)	**Negative FH** mean (SD)	p-value
Number of cases	64	212	-
Age (at registration, in years)	52.1 (7.8)	51.3 (7.8)	0.582
Sex (males)	65.6%	63.7%	0.776
Age at leaving school (years*)	16.6 (1.1)	16.5 (1.0)	0.444
Disease duration (years)	8.7 (7.1)	7.8 (6.5)	0.312
Impaired concentration (s)	68.2%	67.5%	0.968
Impaired memory (s)	72.7%	77.8%	0.466
MOCA test score	25.9 (2.6)	25.3 (3.9)	0.486
Normal (≥ 26)	62.1%	56.9%	0.424
Cognitive impairment (< 26)	37.9%	43.1%	0.424
MCI PD (≥ 20, <26)	35.9 %	37.9 %	0.620
Dementia (< 20)	1.6 %	5.7 %	0.306

FH = family history, * = standard deviation. MCI PD = Mild cognitive impairment Parkinson's disease. Data are presented as mean (standard deviation) except where indicated, * median (inter-quartile range), (s) = self-reported.

Detailed analysis of the MOCA test scores showed significant differences between the 2 sub-groups only in the visuospatial domain (p=0.038) as represented by asking the patients to copy a cube (Table 9.4b). This was also evident in the item of copying 2 intersecting pentagons adapted from the mini mental status exam (p=0.033) (Table 9.4 b)

Table 9.4b is a continuation of Table 9.4a and shows detailed analysis of MOCA test scores of patients (n=276) classified by family history of PD.

Variable	**Positive FH** mean (SD)	**Negative FH** mean (SD)	p-value
Number of cases	64	212	-
MOCA test scores			
Trail	0.94 (0.24)	0.84 (0.37)	0.060
Cube	0.74 (0.44)	0.85 (0.35)	**0.038**
Clock	2.82 (0.43)	2.68 (0.65)	0.246
Pentagons*	0.98 (0.12)	0.90 (0.30)	**0.033**
Naming	2.97 (0.17)	2.94 (0.29)	0.506
Registration	4.76 (0.58)	4.58 (0.95)	0.215
Memory†	3.30 (1.51)	3.05 (1.59)	0.230
Digit span‡	1.86 (0.35)	1.86 (0.39)	0.906
Vigilance	0.95 (0.21)	0.88 (0.32)	0.154
Serial 7's (attention)	2.38 (0.63)	2.31 (0.80)	0.959
Repetition	1.74 (0.54)	1.77 (0.53)	0.701
Lexical fluency	0.61 (0.49)	0.72 (0.45)	0.087
Category fluency* §	23.67 (7.25)	22.84 (7.15)	0.499
Abstraction	1.77 (0.52)	1.64 (0.65)	0.128
Orientation	5.79 (0.41)	5.73 (0.83)	0.581

FH = family history, SD = standard deviation. Data are presented as mean (standard deviation) for comparison with normative data provided by the test publisher, * not part of Montreal cognitive assessment test, †delayed recall, ‡ working memory, §over 90 seconds

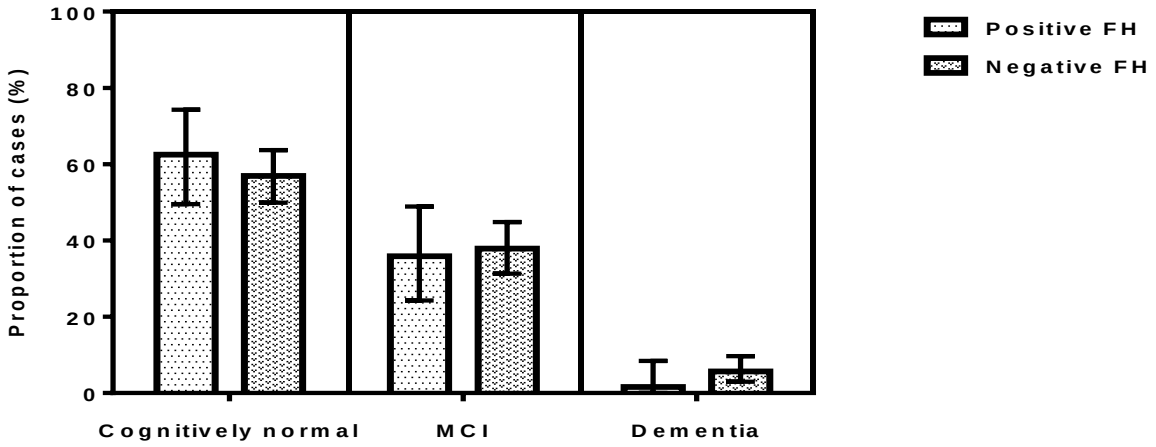

Figure 9.9 shows the proportion of patients who were cognitively normal, had mild cognitive impairment (MCI) or dementia (on the y-axis) as recorded on the MOCA test with respect to their family history (FH) of PD.

9.5 Discussion

Cognitive impairment is common in Parkinson's disease (PD), with long-term longitudinal studies reporting that most patients will develop dementia if they live long enough. The long-term prevalence of dementia in PD from the Sydney multicentre study at 10 years was 38% , 67% at 15 years and 80 % at 20 years in those who were still alive [115]. In the world of dementia, few phrases are more likely to polarise opinion than 'mild cognitive impairment' (MCI) [393]. Some have argued that while cognitive deficits in PD could be due to Lewy body pathology and represent the earliest stages of PD dementia (PDD), but these deficits theoretically could also occur due to dynamic changes in neurotransmitter networks (e.g. dopaminergic pathways) and/or non-PD pathology. As such, defining a PD patient as having MCI (MCI-PD) may not necessarily help in predicting prognosis or clinical course [393]. Others have argued exactly the opposite, that the identification of MCI-PD is important, because it can predict the future development of PDD [394]. Irrespective of the 'baggage' associated with the term MCI and its definition in PD, there seems little doubt that early cognitive impairment in PD, insufficient to merit a diagnosis of PDD, impacts adversely upon patients' self-perceived health status, their ability to undertake activities of daily living and

the strain placed upon their caregivers [395]. MCI-PD is a significant, independent factor contributing to poorer quality of life in patients with newly diagnosed PD. Those classified with greatest impairment (2.0 SD below normal values) have lower quality of life. This has implications for clinical practice and future interventions targeting cognitive impairments [395]. MCI-PD is common in non-demented patients with PD (mean prevalence: 27%; range: 19%–38%) [396] affecting a range of cognitive domains, including memory, visual-spatial, and attention/executive abilities with slightly higher figures emerging from more recent data such as the ICICLE-PD study. This study reported figures of 42.5% even in newly diagnosed patients [397]. In the Norwegian Park West study, using the same criteria i.e. 1.5 standard deviation (SD) below mean for normal healthy controls, this roughly translated to a twofold increase in the proportion with cognitive impairment in subjects with early, untreated PD compared to normal healthy controls [398]. Our study, in the absence of normal healthy controls used the original criteria proposed by the MOCA test authors assigning a cut off value of 26 to define MCI [391] and < 20 to define dementia which have been validated in a PD population [392] , the comparison between groups was adjusted for age, disease duration and educational status . The prevalence of MCI- PD in the EOPD cohort from PRoBaND ranged from 33.5%- 43.8% based on sub-group analysed (Tables 9.1-9.4)) The prevalence of PD dementia in the EOPD cohort from PRoBaND ranged from 0-9.1% based on sub-group analysed (Tables 9.1-9.4)

This variation in the prevalence of cognitive impairment partly reflects differences in disease duration but there is also recognition that there are differences in cognitive impairment between motor subtypes. The PIGD motor subtype is associated with a faster rate of cognitive decline in PD and may be considered a risk factor for incident dementia in PD [386]. Our results replicated this finding and showed the prevalence of cognitive impairment ($p= 0.049$) and dementia ($p=0.013$) were highest in this motor subtype (Table 9.1)

Sub-item analysis of the MOCA test scores showed significant differences were found on five of six domains, specifically visuospatial, executive, language, memory and attention (concentration) with no significant differences in orientation scores (Table 9.2).

MOCA test was used specifically because the alternative instrument, mini mental status examination (MMSE) is handicapped by a lack of adequate representation of executive function tests and these deficits can occur in about a third of patients with PD [399]

Impairments in executive function, attention, visuospatial skills, and memory characterize the "typical" cognitive profile in PD, whereas language and praxis are thought to be relatively spared [400].

In the German Study on Epidemiology of Parkinson's Disease with Dementia (GEPAD) of 873 PD patients there were no differences on the MMSE or the Parkinson Neuropsychometric Dementia Assessment (PANDA) total score between males and females [127], however, subtle differences emerge when more detailed psychometric tests are administered [401-403]. We found no statistically significant differences in the MOCA test scores or the proportion of cases classified as cognitively normal, MCI-PD and demented between the 2 sub-groups.

Sub-item analysis of the MOCA test scores showed significant differences were found in only one of six domains, specifically visuospatial domain (Table 9.2b) with males performing better on the visuospatial task compared to females($p=0.042$).

The age of onset of PD is reported to influence the development of cognitive problems with older age onset significantly associated with the development of dementia [370] . Our results showed no significant differences in the proportion of patients with either mild cognitive impairment ($p=0.325$) or dementia ($p=0.737$) between those who had onset of disease before 40 years of age compared to those who had onset of disease between the ages of 40 and 50 years in the EOPD cohort.

Sub-item analysis of the MOCA test scores also showed no significant differences in any of the six domains assessed (Table 9.3b).

The influence of family history in PD on the onset of dementia has been previously reported. The onset of dementia was significantly earlier in those with no family history of PD compared to those with a family history of PD [404]. Our results showed no statistically significant differences in the MOCA test scores or the proportion of cases classified as cognitively normal, MCI-PD and demented between the 2 sub-groups. Sub-item analysis of the MOCA test scores showed significant differences in only one of six domains, specifically visuospatial domain. Cases with a positive family history of PD performed poorly on the visuospatial task compared to those with sporadic PD ($p=0.038$) (Table 9.2b).

The mechanisms responsible for the variation in the cognitive scores, beyond the effects of age, disease duration and education levels are largely not known. One hypothesis suggests that the heterogeneous cholinergic denervation, in addition to the well-recognised dopaminergic denervation, may account for some of the variability. Previous studies have reported cognitive correlates of cortical cholinergic denervation [405, 406]. On the other hand dopamine levels in frontal regions may influence the fronto-striatal cognitive circuits which may contribute to some of the cognitive deficits. The catechol-O-methyltransferase (COMT) Val (158) Met polymorphism modulates fronto-cortical dopamine turnover in early PD according to a PET study. COMT is the main mode of inactivation for dopamine in frontal areas. Met homozygotes have higher presynaptic dopamine levels in frontal regions than Val homozygotes, which may also help to explain how genotypic variation may influence the fronto-striatal cognitive deficits of PD [407]. Some have investigated brain volumetric correlates of memory in early PD and found right parietal cortical grey matter volume is related to free recall memory deficits [408]. The same group also found striatal volume being related to phonemic verbal fluency in another study [409]. A final piece of evidence for the differences in the biological substrates of PD is that the levels alpha-synuclein, the protein that is central to the degenerative process in PD, measured in the more advanced PD stages decreased in men, but not in women. Further, in men only, plasma alpha-synuclein concentration was associated with cognitive impairments, hallucinations, and sleep disorders in this study underlining the gender-related differences [410]. One could therefore conceptualise that natural variation that might occur in the expression of all these underlying biological substrates could explain at least in part if not all of the variability. Further studies would be required to determine the other so far unexplained determinants of the heterogeneity of cognitive dysfunction in PD.

9.6 Conclusion

Dementia in the late stages of PD is common. MCI-PD almost always precedes the onset of dementia but there is some debate as to whether all those with MCI-PD go on to develop dementia. Nevertheless, recognition of MCI-PD is important but more importantly some phenotypes of PD e.g the PIGD group are even at a greater risk than others. Focussing on this group at its initial stage will enable clinicians to better inform patients about prognosis and to allow these patients the benefits of therapeutic intervention.

Chapter 10. Variation in neuropsychiatric symptoms in EOPD

10.1 Objective

The objective in this chapter is to analyse the variation in the neuropsychiatric and behavioural characteristics and risk factors in a cohort of patients with early onset Parkinson's disease.

10.2 Introduction

Behavioural symptoms such as anxiety and neuropsychiatric problems like depression and psychosis are common in PD. While anxiety and depression can arise de novo and can be part of the premotor syndrome, psychosis and impulse control problems are usually seen as a complication of dopaminergic therapy .These symptoms can sometimes cause more distress to the patents than the motor dysfunction caused by PD. They negatively impact on the quality of life, can cause caregiver distress and may lead to care home placement [411].

10.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0). Data required for determining the prevalence of important depressive screening features such as anhedonia and flat affect and important psychotic phenomena such as delusions and hallucinations were collected from patient filled NMSS at visit 1. Patients also filled in a questionnaire for impulsive-compulsive disorders in Parkinson's disease (QUIP) [412] at visit 1 and the responses were used as a measure of the prevalence of impulse control symptoms

such as overeating, overspending, hyper sexuality and gambling in this cohort. A subjective assessment of patient's perspective whether they believe to suffered from anxiety and depression was determined from responses to relevant questions in the NMSS, this was then more objectively contrasted with the scores obtained on validated Leeds Scales for the Self-Assessment of Anxiety (Leeds SAA) and Depression (Leeds SAD) [413]. Each of these scales has 6 questions with 4 responses and these are scored 1-4. A total score for each scale is obtained by adding the individual scores to each question. Further, those with scores <7 on each scale are classified as normal while those with scores ≥ 7 on the Leeds SAD scale are classified as depressed while those with a score ≥ 7 on the Leeds SAA scale are classified as suffering from anxiety.

In order to analyse the variation in clinical phenotype of PD with respect to historical and objective examination based findings all cases with EOPD were classified according to motor subtypes (TDPD, PIGD, 'Mixed'), gender, age at onset and heritability of the parkinsonian trait as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA).

10.4 Results

276 patients had completed all questionnaires relevant to the behavioural symptoms and 269 of those patients had completed all questionnaires related to the neuropsychiatric symptoms (Figure 10.1). Their demographic details have been presented in Table 5.1 in Chapter 5.

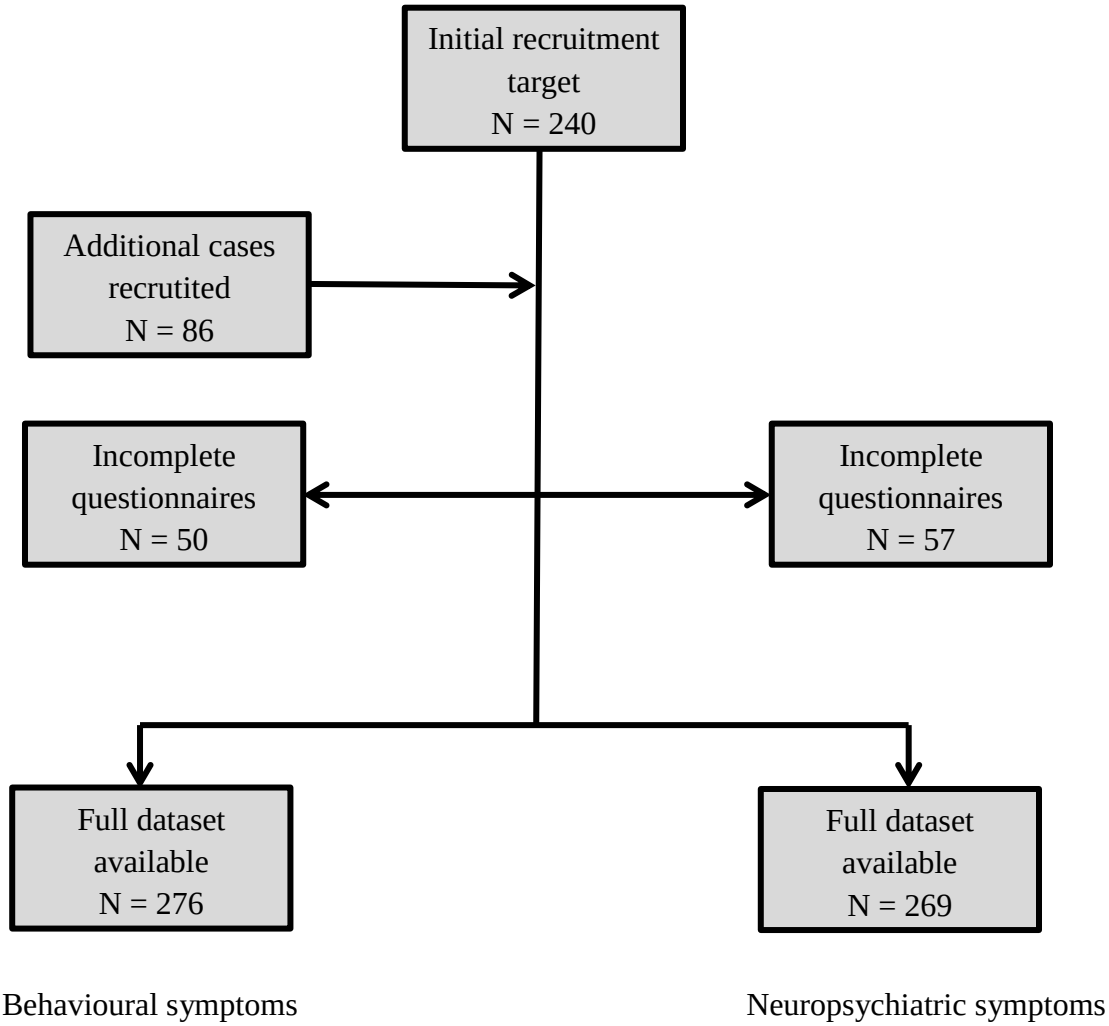

Figure 10.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in the prevalence of neuropsychiatric problems in these patients, classified by motor subtype, is presented in Table 10.1a. There were no statistically significant differences in the prevalence of neuropsychiatric symptoms of anhedonia (Figure 9.2) or delusions (Figure 9.3) between the three motor subtypes.

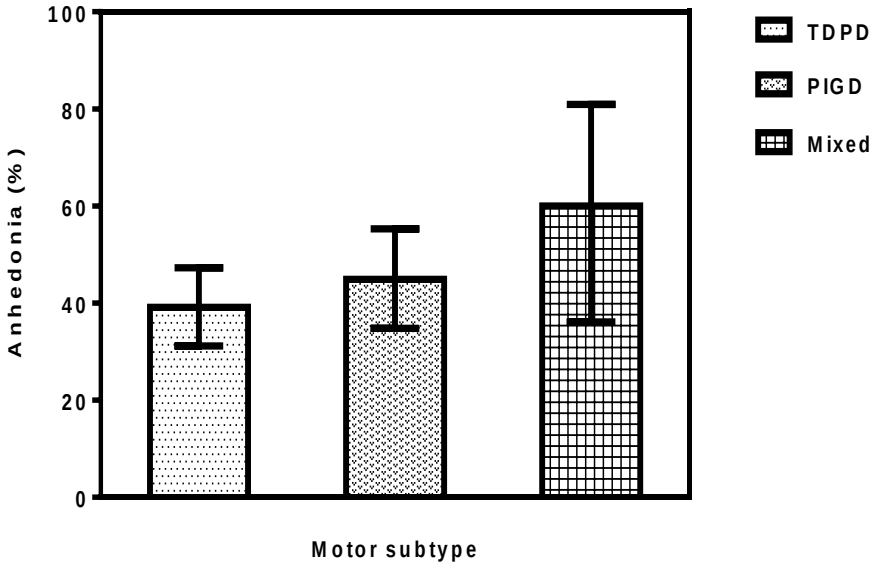

Figure 10.2 shows the proportion of patients who reported anhedonia, using the NMSS questionnaire, (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases reporting anhedonia, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

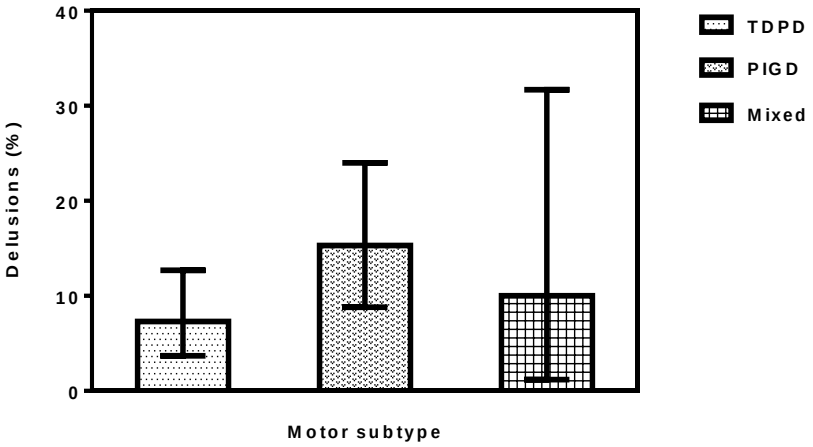

Figure 10.3 shows the proportion of patients who reported delusions, using the NMSS questionnaire, (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases with delusions, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

There were no significant differences in the proportion of cases reporting impulse control problems such as gambling, overeating, overspending, hypersexuality or self-medicating behaviours in between the three groups (Figures 10.4 and 10.5, Table 10.1b)

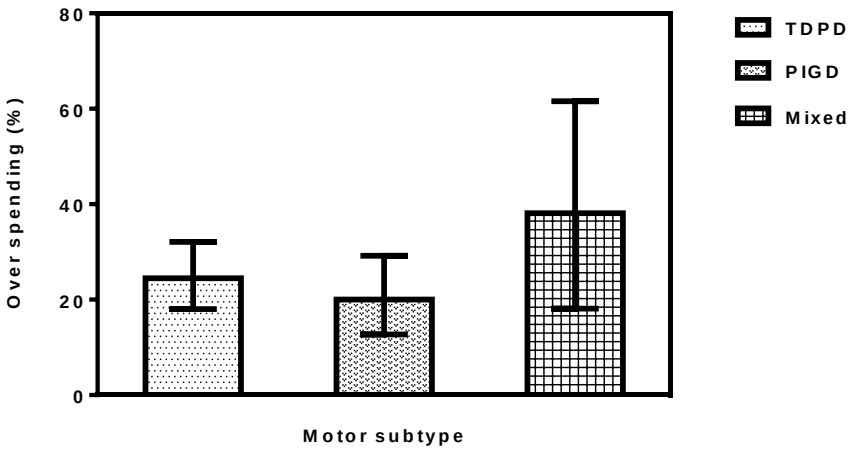

Figure 10.4 shows the proportion of patients who reported overspending, using the QUIP questionnaire, (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases who reported over spending, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion

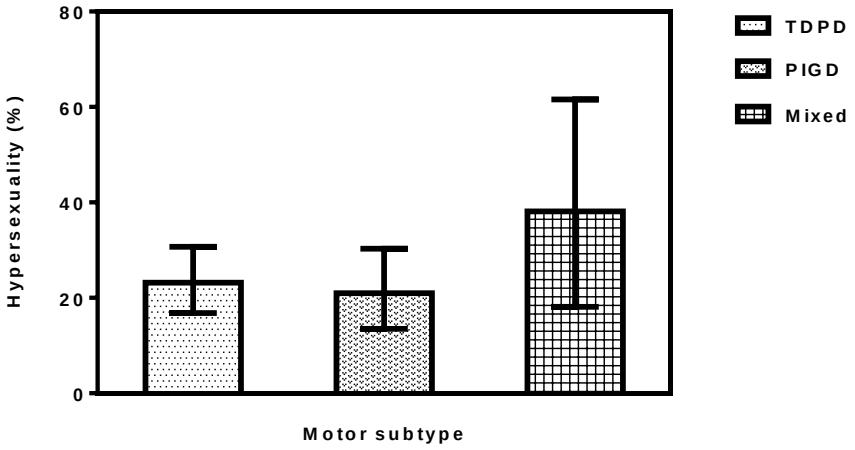

Figure 10.5 shows the proportion of patients who reported hypersexuality, using the QUIP questionnaire, (on the y-axis) and their motor subtype (on the x-axis). The top of the boxes represent the proportion of cases who reported hypersexuality, as indicated on the y-axis, and the whiskers represent the 95% confidence limits of proportion.

Table 10.1a Variation in prevalence of neuropsychiatric symptoms in patients (n=269) classified by motor-subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number of cases	151	98	20	
Age* (in years)	50.4 (45.1-54.2)	54.6 (48.8-59.7)	49.1 (46.8-52.2)	<0.001
Sex (males)	66.9 %	58.2 %	65.0 %	0.373
Duration (years)	4.7 (1.9-9.6)	9.1 (4.6-15.2)	6.5 (2.4-11.8)	<0.001
Anhedonia	39.1 %	44.9 %	60.0 %	0.178
Flat affect	51.7 %	56.1 %	55.0 %	0.781
Delusions	7.3 %	15.3 %	10.0 %	0.129
Hallucinations(visual)	30.5 %	40.8 %	40.0 %	0.218
Anxiety				
Subjective	43.0 %	53.1 %	55.0 %	0.238
Objective †	31.6%	33.6%	12.8%	**0.002**
Depression				
Subjective	59.6%	59.2%	55.0%	0.925
Objective‡	27.4%	29.9%	11.4%	**0.006**

*** Age at registration, IQR = inter-quartile range, † using Leeds SAA general scale for anxiety as follows: normal (0-6), abnormal (≥7); ‡ using Leeds SAD general scale scoring for depression as follows: normal (0-6), abnormal (≥7) [413]**

Post-hoc analysis showed the prevalence of anxiety symptoms using the Leeds SAA general scale was greater in the TDPD compared to the Mixed motor subtype (p=0.001), greater in the PIGD compared to the Mixed motor subtype (p <0.001) but there were no statistically significant differences between TDPD and PIGD subtypes (p=0.692).

Post-hoc analysis showed the prevalence of depressive symptoms using the Leeds SAD general scale was greater in the TDPD compared to the Mixed motor subtype (p=0.001), greater in the PIGD compared to the Mixed motor subtype (p <0.001) but there were no statistically significant differences between TDPD and PIGD subtypes (p=0.692).

Table 10.1b Variation in prevalence of behavioural symptoms in patients (n=269) classified by motor-subtype.

Variable	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed subtype** median (IQR)	**p-value**
Number of cases	155	100	21	
Age***** (in years)	50.4 (45.1-54.3)	54.6 (48.8-59.8)	49.6 (46.9-52.5)	**<0.001**
Sex (males)	67.7 %	59 %	65 %	0.356
Duration (years)	5.1 (2.0-9.6)	9.1 (4.6-15.0)	7.4 (2.5-11.8)	**<0.001**
Over-eating	17.4 %	21.0 %	19.0 %	0.775
Over-spending	24.5 %	20.0 %	38.1 %	0.203
Hypersexuality	23.2 %	21.0 %	38.1 %	0.242
Gambling	16.8 %	22.0 %	33.3 %	0.165
Self-medication	5.8 %	10.0 %	4.8 %	0.407
DA (%)	63.9 %	63.0 %	42.9 %	0.171
LEDD (mg/day)	599 (300-965)	840 (532-1043)	738 (538-1053)	**0.003**

*** Age at registration, IQR = inter-quartile range, DA = dopamine agonists, LEDD = levodopa equivalent daily dose**

There were no differences in any of the neuropsychiatric symptoms subjectively or objectively (Table 10.2a, Figures 10.6 and 10.7) between males and females.

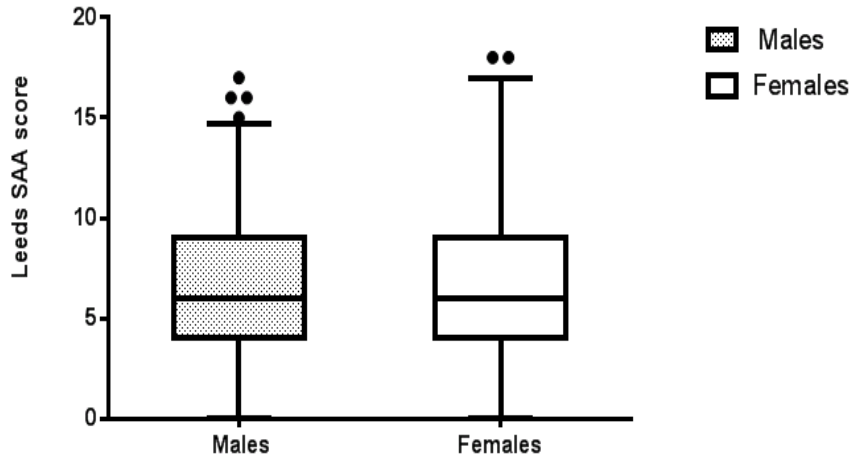

Figure 10.6 shows the Leeds SAA scale score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between males (n=171) and females (n=98) (p=0.442).

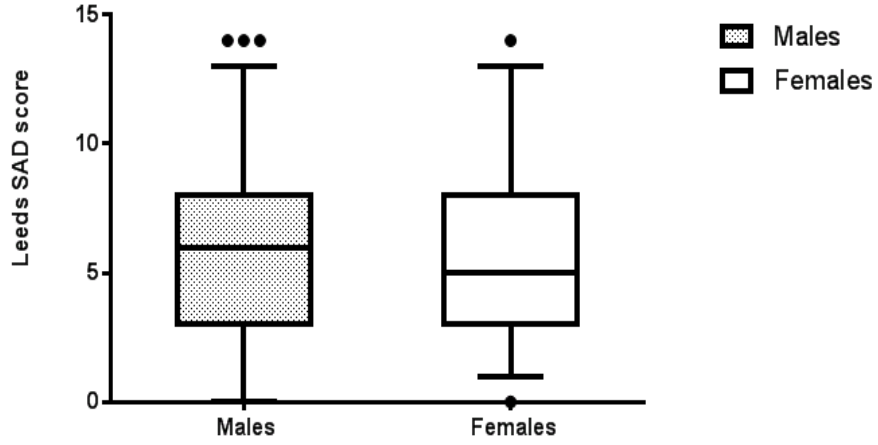

Figure 10.7 shows the Leeds SAD scale score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between males (n=171) and females (n=98) (p=0.988).

Table 10.2a Variation in prevalence of neuropsychiatric symptoms in patients (n=269) classified by gender.

Variable	**Males median (IQR)**	**Females median (IQR)**	**p-value**
Number of cases	171	98	-
Age* (years)	51.4 (46.2-56.4)	51.0 (47.2-55.5)	0.945
Disease duration (years)	7.3 (2.7-11.5)	6.1 (2.5-12.2)	0.852
Anhedonia	45.6 %	37.8 %	0.209
Flat affect	53.2 %	67.4 %	0.891
Delusions	10.5 %	10.2 %	1.000
Hallucinations (visual)	36.3 %	32.7 %	0.596
Anxiety			
Subjective	48.6%	52.5%	0.530
Objective†	35.1%	30.5%	0.351
Depression			
Subjective	56.5%	65.7%	0.136
Objective‡	32.0%	25.8%	0.194

*** Age at registration, IQR = inter-quartile range, † using Leeds SAA general scale for anxiety as follows: normal (0-6), abnormal (≥7); ‡ using Leeds SAD general scale scoring for depression as follows: normal (0-6), abnormal (≥7) [413].**

The were no significant differences in the proportion of cases reporting impulse control problems such as gambling, overeating, overspending, hypersexuality or self-medicating behaviours in between the two groups (Table 10.2b).

Table 10.2b Variation in prevalence of behavioural symptoms in patients (n=269) classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p-value**
Number of cases	177	99	-
Age * (years)	51.7 (46.5-56.8)	51.1 (47.2-55.6)	0.968
Disease duration (years)	7.3 (2.8-11.5)	6.5 (2.6-11.9)	0.737
Over-eating	19.8 %	17.2 %	0.937
Over-spending	23.2 %	25.3 %	0.696
Hypersexuality	15.9 %	13.1 %	0.535
Gambling	21.5 %	13.1 %	0.087
Self-medication	9.0 %	4.0 %	0.124
DA (%)	58.2 %	68.7 %	0.085
LEDD (mg/day)	760 (400-1030)	600 (328-986)	0.136

*** Age at registration, IQR = inter-quartile range, DA = dopamine agonists, LEDD = levodopa equivalent daily dose.**

There were no differences in any of the neuropsychiatric symptoms (Table 10.3a) subjectively or objectively (Figures 10.8 and 10.9), between those diagnosed with PD before the age of 40 years and those diagnosed with PD after the age of 40 years except the prevalence of anxiety, objectively recorded using the Leeds SAA scale, was greater in those diagnosed with PD after the age of 40 years (p=0.027).

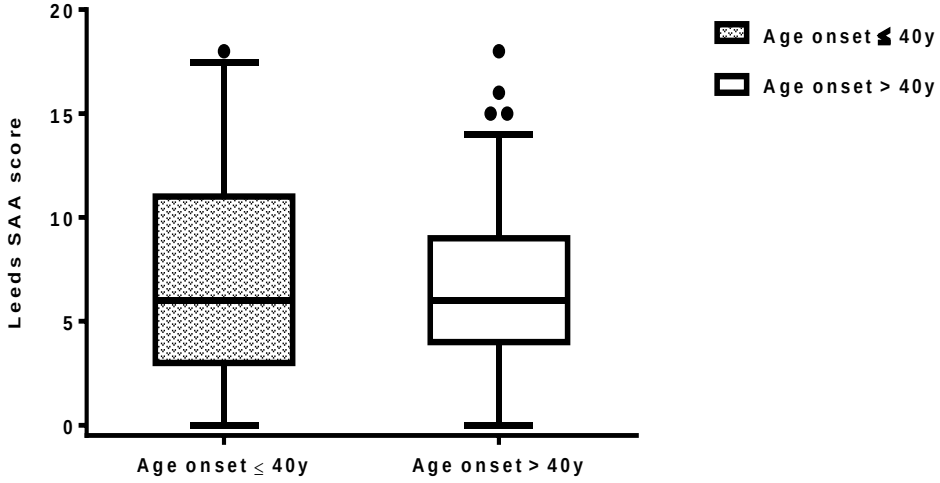

Figure 10.8 shows the Leeds SAA scale score (on the y-axis) and the age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with age at onset of PD ≤ 40 years (n=63) compared to those with age at onset of PD > 40 years (n=206) (p=0.737).

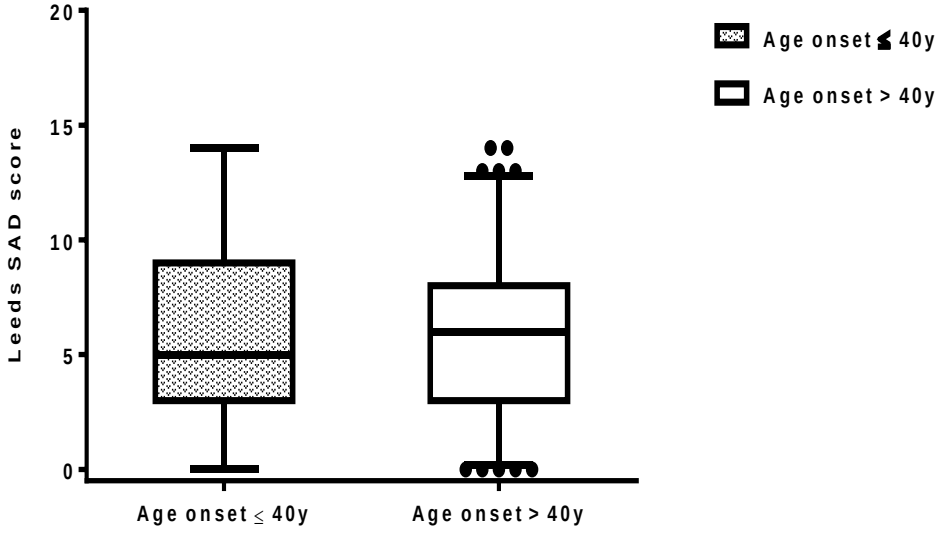

Figure 10.9 shows the Leeds SAD scale score (on the y-axis) and the age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with age at onset of PD ≤ 40 years (n=63) compared to those with age at onset of PD > 40 years (n=206) (p=0.539).

Table 10.3a Variation in prevalence of neuropsychiatric symptoms in patients (n=269) classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40yrs** median (IQR)	**p-value**
Number of cases	63	206	-
Age* (in years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	<0.001
Sex (males)	67.2%	63.2%	0.559
Disease duration (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.049
Anhedonia	50.8 %	40.3 %	0.140
Flat affect	61.9 %	51.0 %	0.127
Delusions	12.7 %	9.7 %	0.486
Hallucinations (visual)	34.9 %	45.6 %	0.133
Anxiety			
Subjective	50.8 %	46.6 %	0.559
Objective†	25.4 %	37.0 %	**0.027**
Depression			
Subjective	60.3 %	58.7 %	0.823
Objective‡	22.9 %	32.6 %	0.055

*** Age at registration, IQR = interquartile range,† using Leeds SAA general scale for anxiety as follows: normal (0-6), abnormal (≥7); ‡ using Leeds SAD general scale scoring for depression as follows: normal (0-6), abnormal (≥7) [413].**

The were no significant differences in the proportion of cases reporting impulse control problems such as gambling, overeating , overspending or self-medicating behaviours in between the two groups. The only significant difference was that about one third of patients diagnosed with PD before the age of 40 years reported hyper sexuality compared to about one

fifth of those diagnosed with PD after the age of 40 years in the EOPD cohort (p=0.046) (Table 10.3 b)

Table 10.3b Variation in subjective and objective assessment of neuropsychiatric symptoms in patients (n=269) classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs**	**Age onset > 40 yrs**	**p- value**
Number of cases	64	212	-
Age* (in years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	**<0.001**
Sex (males)	67.2%	63.2%	0.561
Disease duration (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.707
Over-eating	21.9 %	17.9 %	0.479
Over-spending	23.4 %	24.1 %	0.919
Hypersexuality	32.8 %	20.8 %	**0.046**
Gambling	21.9 %	19.3 %	0.721
Self-medication	12.5 %	5.7 %	0.094
DA (%)	60.9 %	62.3 %	0.848
LEDD (mg/day)	700 (363-998)	715 (400-1008)	0.659

*** Age at registration, IQR = interquartile range, DA = dopamine agonists, LEDD = levodopa equivalent daily dose**

There were statistically significant differences in the prevalence of anxiety (p=0.022) and depression (p=0.014) recorded objectively using the Leeds SAA and SAD scales (Figures 10.10 and 10.11), in between those cases with a positive family history PD compared to those with negative family history of PD, both symptom complexes being more prevalent in those with sporadic PD i.e. no family history of PD (Table 10.4a).

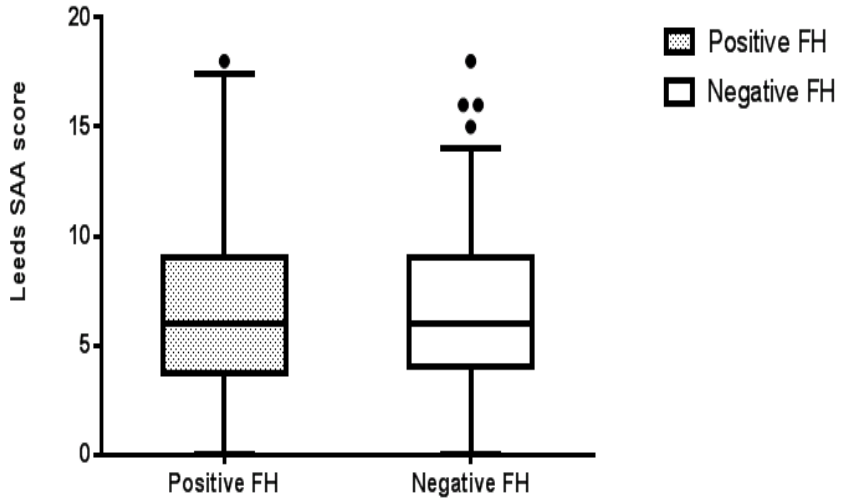

Figure 10.10 shows the Leeds SAA scale score (on the y-axis) and the family history of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with familial PD (n=63) compared to those with sporadic PD (n=206) (p=0.664).

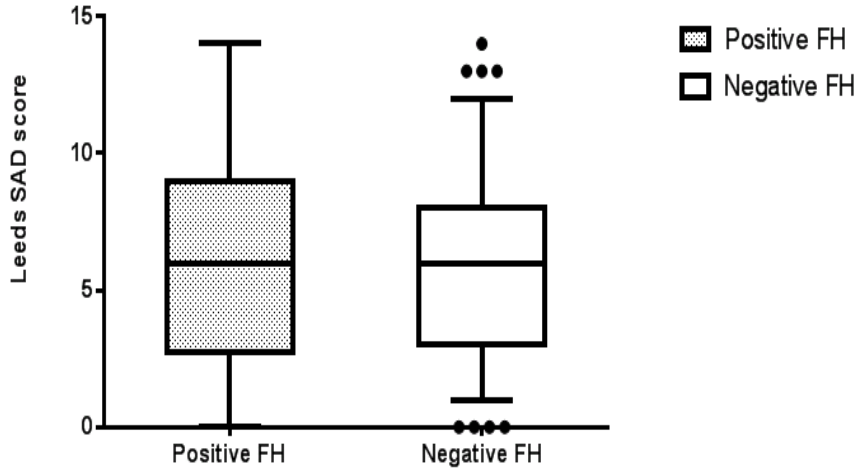

Figure 10.11 shows the Leeds SAD scale score (on the y-axis) and the family history of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with familial PD (n=63) compared to those with sporadic PD (n=206) (p=0.899).

Table 10.4a Variation in prevalence of neuropsychiatric symptoms in patients (n=269) classified by family history of PD.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	63	206	-
Age *(in years)	52.3 (47.4-56.8)	51.0 (46.9-55.7)	0.296
Sex (males)	65.1%	63.1%	0.776
Disease duration (years)	6.9 (3.5-12.3)	6.7 (2.3-11.3)	0.239
Anhedonia	42.9 %	42.7 %	0.985
Flat affect	54.0 %	53.4 %	0.937
Delusions	9.5 %	10.7 %	1.000
Hallucinations (visual)	34.9%	33.0%	0.779
Anxiety			
Subjective	50.8 %	46.6 %	0.559
Objective†	25.0%	36.9%	**0.022**
Depression			
Subjective	57.1 %	59.7 %	0.717
Objective‡	21.0%	33.5%	**0.014**

FH = family history, * Age at registration, IQR= inter-quartile range. Data are presented as median (inter-quartile range) except where indicated. † using Leeds SAA general scale for anxiety as follows: normal (0-6), abnormal (≥7); ‡ using Leeds SAD general scale scoring for depression as follows: normal (0-6), abnormal (≥7) [413].

The were no significant differences in the proportion of cases reporting impulse control problems such as gambling, overeating , overspending or self-medicating behaviours in between the two groups (Table 10.4b).

Table 10.4b Variation in prevalence of behavioural symptoms in patients (n=269) classified by family history of PD.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	63	206	-
Age *(in years)	52.3 (47.4-56.8)	51.0 (46.9-55.7)	0.582
Sex (males)	65.1%	63.1%	0.776
Disease duration (years)	6.9 (3.5-12.3)	6.7 (2.3-11.3)	0.312
Over-eating	18.8 %	18.9 %	0.983
Over-spending	23.4 %	24.1 %	0.125
Hyper sexuality	29.7 %	21.7 %	0.187
Gambling	18.8 %	20.3 %	0.859
Self-medication	3.1 %	8.5 %	0.178
DA (%)	60.9 %	62.3 %	0.848
LEDD (mg/day)	824 (460-1033)	655 (400-1000)	0.135

FH = family history, * Age at registration, IQR = interquartile range, DA = dopamine agonists, LEDD = levodopa equivalent daily dose

10.5 Discussion

Using rigid criteria such as DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition) may miss a substantial number of cases with neuropsychiatric symtpoms [414], particularly those with a sub-syndromal diagnosis, who may end up not being treated even when symptoms are significantly affecting quality of life, therefore a more inclusive approach is advocated, nevertheless some objective measures and clinimetric scales to quantify the prevalence and severity of these problems in the PD population are are required for practical purposes. There are several scales that can be used for each diagnostic category. The choice of scale depends on several factors like ease of use, applicability to the population in question and the research question being answered. The scales for depression and anxiety

i.e. Leeds SAA and SAD scales used in this chapter had been chosen as these are validated instruments that are used in similar large scale epidemiological studies such as the Oxford Parkinson disease centre (OPDC) discovery cohort such that datasets could be shared for cross-comparisons and meta-analysis, while recognising the fact that other research groups have used different scales.

The influence of the motor subtype on neuropsychiatric features such as depression and mood has been investigated, with the PIGD subtype reported to have a greater association with depression compared to TDPD [415] but other studies such as the PROMS-PD study found no statistically significant difference in the proportion of patients classified as depressed or anxious using the HADS scales [416]. Our results, however, showed the prevalence of depression using the Leeds SAD scale was greater in both TDPD ($p = 0.001$) and PIGD ($p < 0.001$) compared to the 'Mixed' motor subtype but no significant differences were found between the TDPD and PIGD subtypes ($p=0.692$). Using the Leeds SAA scale, we also found the prevalence of depression using the Leeds SAD scale was greater in both TDPD ($p=0.001$) and PIGD ($p < 0.001$) compared to the 'Mixed' motor subtype but no significant differences were found between the TDPD and PIGD subtypes ($p=0.692$).

In one previous study of neuropsychiatric symptoms in PD gender did not seem to have an influence on the prevalence of symptoms in the mood and apathy clusters from the NPI [417] but another study, using GDS found the female gender to be significantly associated with depressive symptoms [418]. Our results showed no statistically significant differences in the prevalence of either anxiety or depressive symptoms both subjectively and objectively between the two genders (Table 10.2a).

The age of onset of PD is reported to influence the development of behavioural and neuropsychiatric symptoms with old-age onset significantly associated with the development of hallucinations, loss of interest and impaired concentration [370]. Our results also indicated a greater proportion of patients diagnosed with PD after the age of 40 years to be affected with depression, using the Leeds SAD scale, compared to those diagnosed with PD before the age of 40 years ($p=0.027$) but there was no significant difference in the proportion of patients diagnosed with anxiety using the Leeds SAA scale between the two groups ($p=0.055$). One

could hypothesize that the biologic mechanisms for these two different, yet overlapping, set of symptoms may be different.

The association of neuropsychiatric features such as depression in PD with a positive family history of PD is reported [56]. Our study, however, found a greater prevalence of anxiety (p=0.014) and depression (p=0.022) objectively in those with a negative family history of PD (sporadic PD) compared to those with a positive family history of PD despite there being no statistically differences in the age and disease duration between the 2 groups (Table 10.4a).

There were no significant differences in the proportion of cases reporting impulse control problems such as gambling, overeating, overspending or self-medicating behaviours in between cases classified by motor subtype (Table 10.1b), gender (Table 10.2b), age at onset of PD (Table 10.3b) and hereditary versus sporadic PD (Table 10.4b). This is in keeping with previous studies showing no relationship between motor phenotypes and impulse control behaviour symptoms [419]. The only significant differences noted, in our analysis, were in the proportion of patients reporting hyper sexuality between those with age at diagnosis of PD less than 40 years (30.8%) compared to those with age at diagnosis of PD greater than 40 years (20.8%) (p=0.046), even though there was no significant difference in the proportion of cases on dopamine agonists in the two groups (p=0.848).

The mechanisms leading to neuropsychiatric problems and behavioural symptoms remain largely unknown however there has been some evidence to suggest that both sets of problems are associated with aberrations in ventral striatal dopamine signalling and concomitant dysfunction of the limbic cortico-striatal-thalamocortical circuit. Depression in PD seems associated with decreased activity in the limbic cortico-striatal-thalamo-cortical circuit, whereas impulse control problems seem associated with increased limbic cortico-striatal-thalamo-cortical circuit activity, usually after commencing dopamine replacement therapy [420]. Besides the role of monoamine neurotransmitter circuits, there is evidence from other sources of possible contribution of the cholinergic system and white-matter change, in older depressed patients [416]. The determinants of psychosis in a study of 755 PD patients were a combination of age, disease duration, H&Y stage and medication (dopamine agonists and COMT inhibitors) usage [421].

10.6 Conclusion

The variation in the underlying biological substrates and environmental influences such as drug side effects could account for some of the variability in the neuropsychological profiles in the subgroups of PD, however, more work is required to explain other possible determinants of this heterogeneity.

Chapter 11. Variation in QoL and determinants of QoL in EOPD

11.1 Objective

The objective in this chapter is to analyse the variation in the quality of life (QoL) scores and its determinants in a cohort of patients with early onset Parkinson's disease.

11.2 Introduction

Measurement of quality of life has become increasingly relevant as an outcome parameter in almost all clinical trials of PD [422]. Quality of life is a multidimensional concept that in the broadest sense encompasses a subjective evaluation by a person of his or her satisfaction with life and concerns, among others, the relationships with family or relatives, the person's own health, the health of another close person, finances, housing, independence, religion, social life, and leisure activities [423]. Health and independence in activities of daily living contribute to quality of life, and this domain is often referred to as health related quality of life which we will abbreviate as QoL.

11.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0). Patients filled in questionnaires about their independence in activities of daily living (ADL) using Schwab and England scale [424] and Parkinson's disease quality of life eight items (PDQ-8) [130] at study visit 1. The final scores obtained on PDQ-8 were converted into a PDQ8 summary index (PDQ8SI) score by using the following formula PDQ8 score/32 x100. Data required for determining the motor and non-motor symptoms that impact on quality of life (QoL) were assimilated from UPDRS scores, NMSS and their HY stage documented at the same study visit [425].

In order to analyse the variation in motor complications of PD with respect to historical and examination findings all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed'), gender, age at onset of PD symptoms and heritability of the Parkinsonian trait as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes (pairwise comparisons between groups were made adjusting for multiple comparisons with Dunn's correction). Post hoc comparisons between groups were done using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

As a first step, the factors that would be used in generalised linear models, deemed to have an impact on the PDQ8SI, would be determined by correlation analysis, using Spearman's correlation coefficients.

Generalized linear models, based on ANCOVA, with age, disease duration, UPDRS3 scores, HY stages, NMSS scores and ADL scores as covariates were employed to determine any significant differences in subgroups classified by motor subtype, gender, age at onset and hereditary versus sporadic parkinsonism (independent variables) on quality of life scores (dependent variable). Log10 transformations were used where data distributions failed the Shapiro Wilk test of normality.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (*version 23 for Windows*; IL, USA).

11.4 Results

269 patients had completed all questionnaires relevant to this analysis and are included here (Figure 11.1). Their demographic details are presented in Tables 11.2 to 11.5.

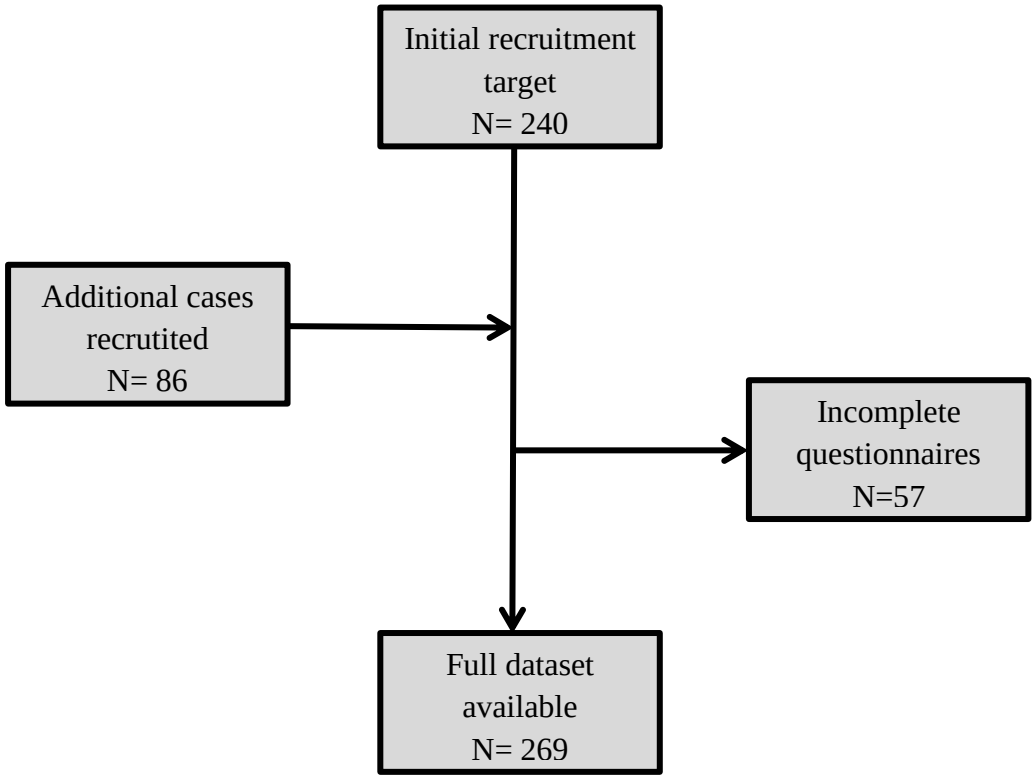

Figure 11.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The factors that were deemed to have an impact on the quality of life scores (PDQ8SI), determined by correlation analysis, using Spearman's correlation coefficients are shown in Table 11.1.

Table 11.1 Factors that had an influence on the quality of life scores in patients.

Variable	**Median (IQR) n=269**	**Correlation with PDQ8SI (Spearman's rho)**	**p-value**
Age (years)	51.5 (46.9-56.5)	0.217	**<0.001**
Disease duration (years)	6.8 (2.7-11.5)	0.351	**<0.001**
UPDRS1	16 (14-18)	0.482	**<0.001**
UPDRS2	15 (9-22)	0.740	**<0.001**
UPDRS3	24 (15-37)	0.449	**<0.001**
UPDRS4	2 (0-7)	0.486	**<0.001**
HY stage	2.0 (1.5-2.5)	0.441	**<0.001**
NMSS score	39 (24-71)	0.707	**<0.001**
ADL	80 (60-90)	-0.577	**<0.001**

UPDRS = Movement disorder society (MDS) unified Parkinson's disease rating scale, UPDRS1 = Part 1 of the MDS-UPDRS scale, UPDRS2 = Part 2 of the MDS-UPDRS scale, UPDRS3= Part 3 of the MDS-UPDRS scale, UPDRS4 = Part 4 of the MDS-UPDRS scale, HY = modified Hoehn Yahr scale, NMSS = non- motor symptoms scale for PD, ADL = independence in activities of daily living recorded on the Schwab and England scale

The variation in the activities of daily living (Figure 11.2), the quality of life scores (Figure 11.3 and 11.4) and the factors that influence the quality of life in these patients classified by motor subtype are presented in Tables 11.1. There were statistically significant differences in the quality of life scores between the three motor subtypes (Table 11.2).

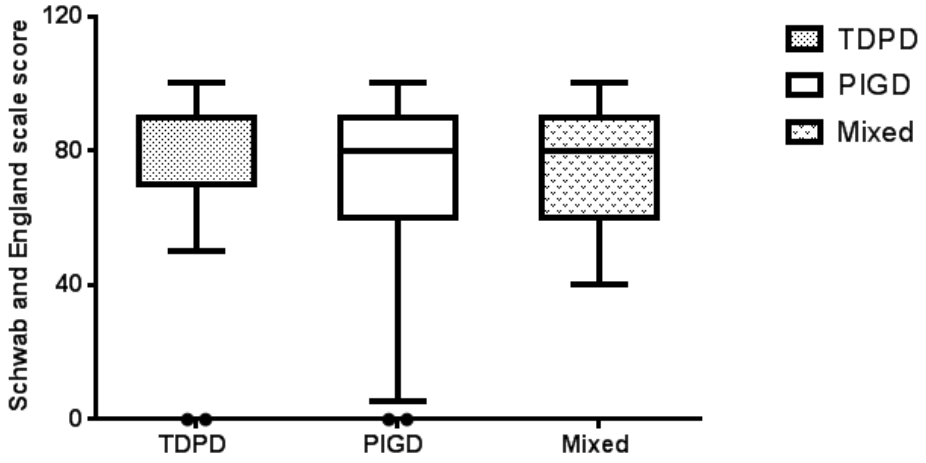

Figure 11.2 shows the Schwab and England (SE) scale score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. SE scale scores were greater in those with TDPD (n=151) compared to those with PIGD (n=98) ($p<0.001$).

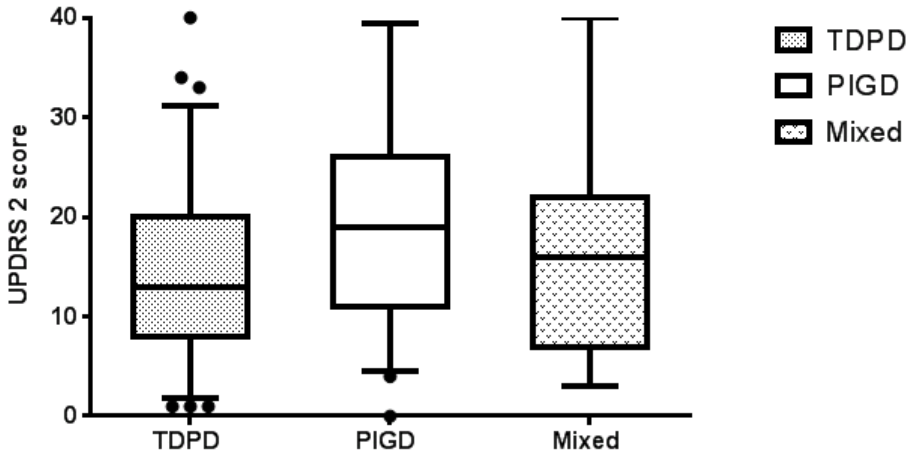

Figure 11.3 shows the movement disorder society unified Parkinson's disease Part 2 (UPDRS 2) score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. UPDRS 2 scores were greater in those with PIGD (n=98) compared to those with TDPD (n=151) ($p=0.028$).

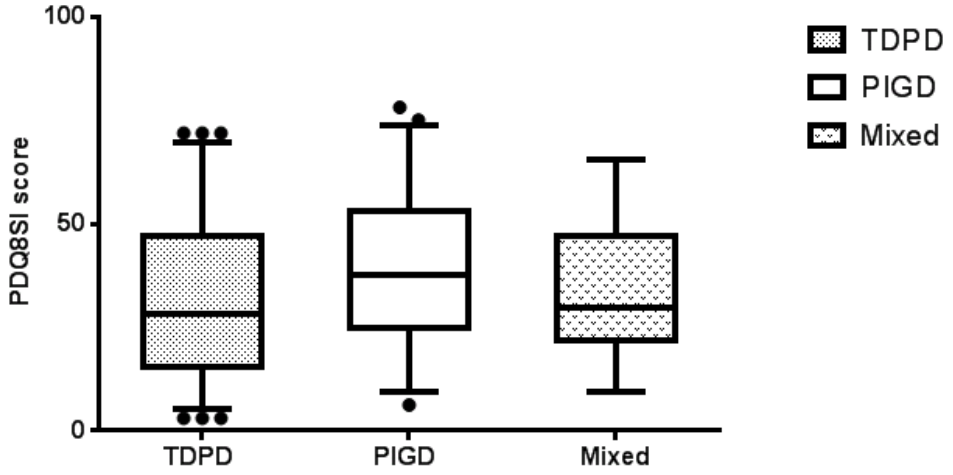

Figure 11.4 shows the Parkinson's disease quality of life summary index (PDQ8SI) score (on the y-axis) and the motor subtype (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No significant differences were found between those with TDPD (n=151), PIGD (n=98) and 'Mixed' motor subtypes (n=20) (p=0.796).

11.2 Variation in the activities of daily living (ADL), quality of life (QoL) scores and determinants of QoL in patients (n=269) classified by motor subtype.

Variable/ Scale	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed subtype** median (IQR)	**p- value**
Number of cases	151	98	20	-
Age* (years)	50.4 (45.1-54.2)	54.6 (48.8-59.7)	49.1 (46.8-52.2)	<0.001
Sex (males)	66.9 %	58.2 %	65.0 %	0.373
Duration (years)	4.7 (1.9-9.6)	9.1 (4.6-15.2)	6.5 (2.4-11.8)	<0.001
UPDRS score				
UPDRS Part 1	16 (14-17)	17 (14-20)	16 (14-17)	0.924
UPDRS Part 2	13 (8-20)	19 (11-26)	16 (8-21)	**0.028**
UPDRS Part 3	22 (14-36)	28 (19-38)	15 (10-22)	**0.007**
UPDRS Part 4	2 (0-6)	5 (2-10)	4 (0-10)	0.452
Hoehn Yahr stage	2.0 (1.0-2.0)	2.0 (2.0-3.0)	1.5 (1.0-2.0)	<0.001¥
NMSS score				
Autonomic	10 (4-18)	12 (5-22)	12 (8-19)	0.486
Sleep/fatigue	8 (4-16)	12 (4-21)	7 (3-14)	0.140
Mood/apathy	4 (1-10)	4 (1-14)	11 (2-17)	0.132
Percept. /halluc.	0 (0-1)	1 (0-3)	0 (0-1)	0.073
Attention/memory	3 (1-8)	4 (2-8)	5 (2-6)	0.843
Total score	36 (21-65)	48 (25-77)	43 (26-84)	0.433
ADL score	90 (75-90)	80 (60-90)	85 (60-90)	**<0.001**
PDQ8SI score	29.7 (15.6-46.9)	37.5 (25.0-53.1)	31.3 (21.9-46.9)	0.796

*** Age at registration, IQR = inter-quartile range, UPDRS = Movement disorder society unified Parkinson's disease rating scale, NMSS = non motor symptoms scale, PDQ8SI = Parkinson's disease quality of life summary index, ADL = activities of daily living using Schwab and England scale**

Post hoc tests showed the difference in the UPDRS2 scores was between TDPD and PIGD (P<0.001) with PIGD subtype having higher scores but not between TDPD and 'Mixed' (p=0.526) or between PIGD and 'Mixed' (p=0.114).

There were also significant differences in the UPDRS 3 scores between PIGD and 'Mixed' (p=0.004) with PIGD subtype having the higher scores, between TDPD and 'Mixed' (p=0.035) with TDPD having the higher scores but there were no significant differences between TDPD and PIGD (p=0.055) UPDRS3 scores on post hoc tests.

Further, post hoc tests showed the differences in ADL scores between TDPD and PIGD (p<0.001) with TDPD having higher scores but no significant differences between PIGD and 'Mixed' (p=0.213) as well as between TDPD and 'Mixed' (p=0.188).

Classifying patients by gender, however, showed no differences in either the activities of daily living (Figure 11.5) or quality of life scores (Figures 11.6 and 11.7, Table 11.3).

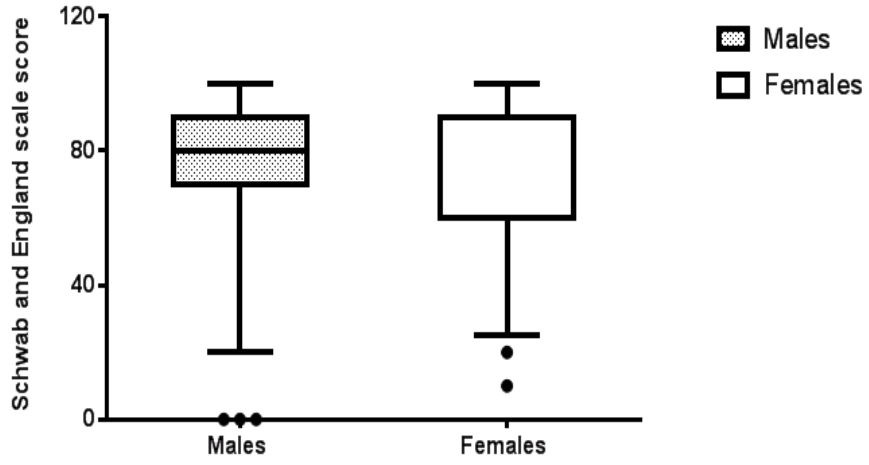

Figure 11.5 shows the Schwab and England scale score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between males (n=171) and females (n=98) (p=0.987).

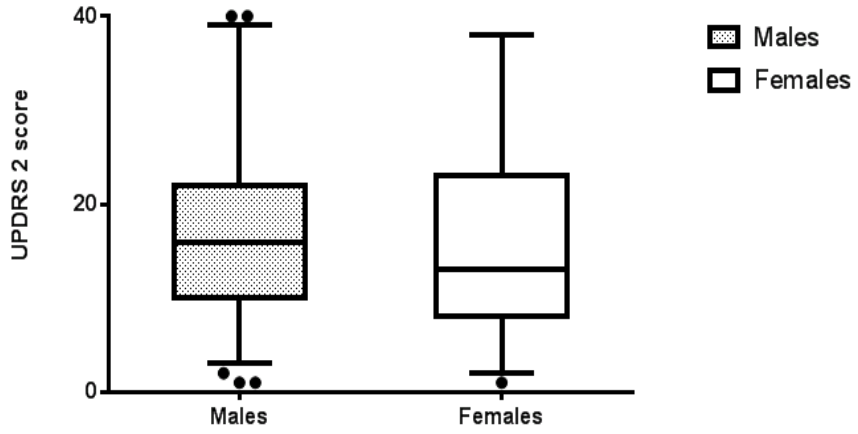

Figure 11.6 shows the movement disorder society unified Parkinson's disease part 2 (UPDRS 2) score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between males (n=171) and females (n=98) (p=0.152).

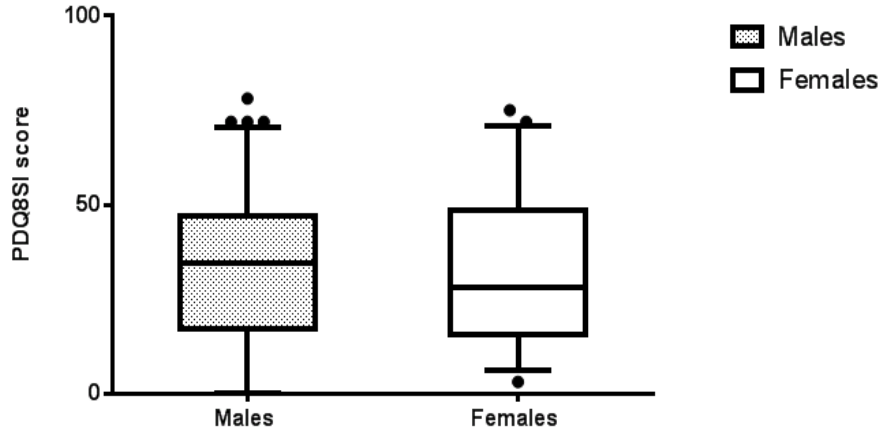

Figure 11.7 shows the Parkinson's disease quality of life summary index (PDQ8SI) score (on the y-axis) and the gender (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between males (n=171) and females (n=78) (p=0.213).

11.3 Variation in the activities of daily living (ADL), quality of life (QoL) scores and determinants of QoL in patients (n=269) classified by gender.

Variable/ Scale	**Males** median (IQR)	**Females** median (IQR)	**p- value**
Number of cases	171	98	-
Age* (in years)	51.4 (46.2-56.4)	51.0 (47.2-55.5)	0.945
Duration (years)	7.3 (2.7-11.5)	6.1 (2.5-12.2)	0.852
UPDRS score			
UPDRS Part 1	16 (14-19)	15 (14-17)	0.335
UPDRS Part 2	16 (10-22)	13 (8-23)	0.152
UPDRS Part 3	24 (15-36)	23 (14-36)	0.456
UPDRS Part 4	3 (0-8)	4 (0-8)	0.842
Hoehn Yahr stage	2.0 (1.5-2.5)	2.0 (1.0-2.5)	0.566
NMSS score			
Autonomic	11 (4-19)	10 (5-18)	0.958
Sleep/fatigue	8 (4-17)	11(4-17)	0.391
Mood/apathy	4 (1-13)	4 (1-12)	0.580
Percept. /halluc.	0 (0-2)	0 (0-2)	0.858
Attention/memory	4 (2-8)	3 (1-7)	0.118
Total score	41 (24-70)	36 (24-73)	0.743
ADL score	80 (70-90)	90 (60-90)	0.987
PDQ8SI score	34.4 (17.2-46.9)	29.7 (15.6-47.7)	0.213

*** Age at registration, IQR= interquartile range. Data are presented in median (inter-quartile range) except where indicated. UPDRS= Movement disorder society unified Parkinson's disease rating scale, NMSS= non motor symptoms scale, PDQ8SI= Parkinson's disease quality of life summary index, ADL=activities of daily living using Schwab and England scale**

There was no differences in either the activities of daily living (Figure 11.8) or the quality of life scores (Figure 11.9 and 11.10) in those diagnosed with PD before the age of 40 years compared to those diagnosed after the age of 40 years (Table 11.4).

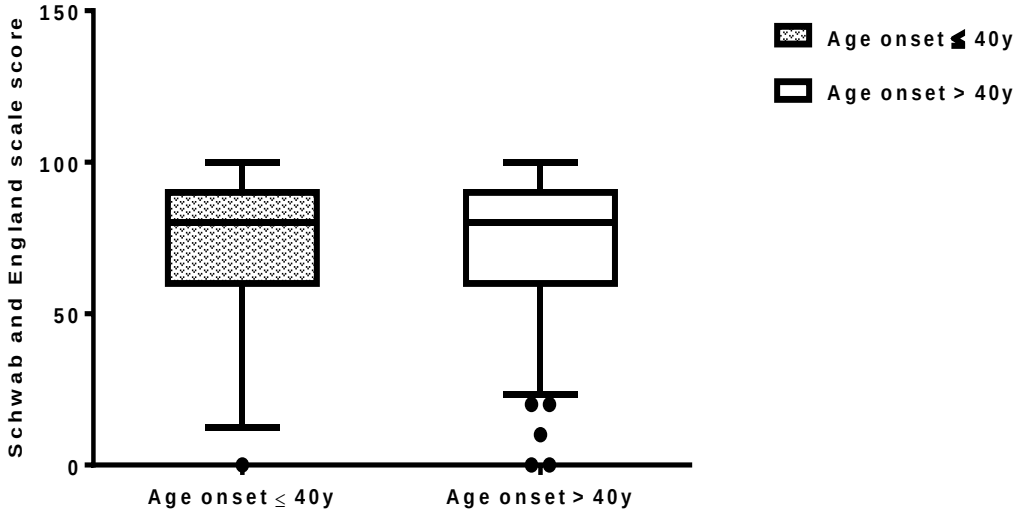

Figure 11.8 shows the Schwab and England scale score (on the y-axis) and the age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with age at onset of PD ≤ 40 years (n=63) compared to those with age at onset of PD > 40 years (n=206) (p=0.095).

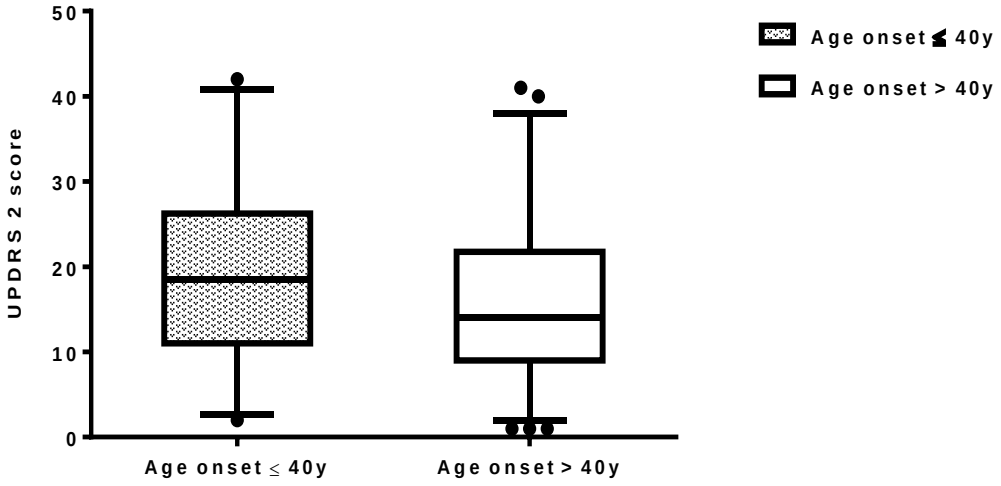

Figure 11.9 shows the movement disorder society unified Parkinson's disease part 2 (UPDRS 2) score (on the y-axis) and the age at onset of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with age at onset of PD ≤ 40 years (n=63) compared to those with age at onset of PD > 40 years (n=206) (p=0.315).

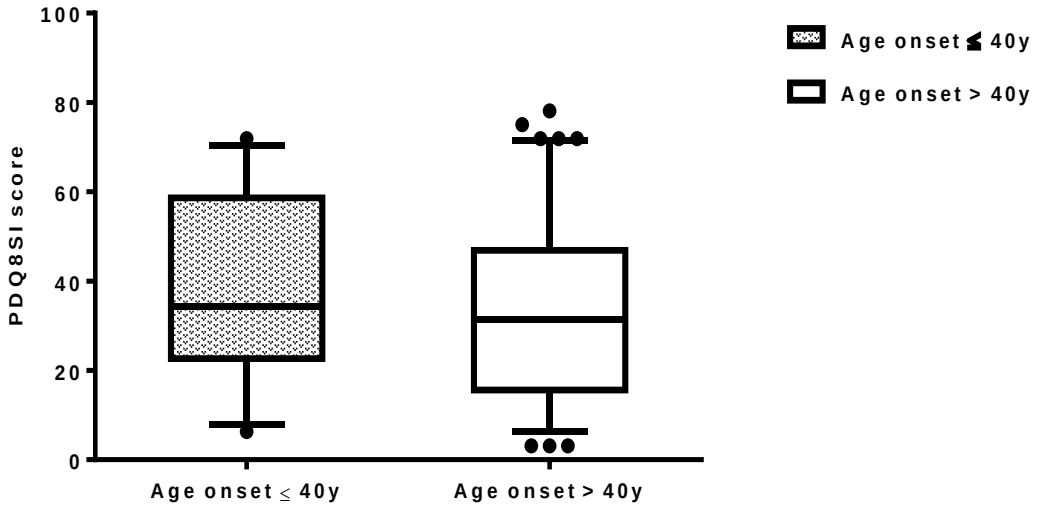

Figure 11.10 shows the Parkinson's disease quality of life summary index (PDQ8SI) score (on the y-axis) and the age at onset of PD (on the x-axis) of the patients (n=276) in the EOPD cohort of the PRoBaND study. The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with age at onset of PD ≤ 40 years (n=63) compared to those with age at onset of PD > 40 years (n=206) (p=0.285).

11.4 Variation in the activities of daily living (ADL), quality of life (QoL) scores and determinants of QoL in patients (n=269) classified by age at onset of PD.

Variable/ Scale	**Age onset ≤ 40 yrs.** median (IQR)	**Age of onset > 40 yrs.** median (IQR)	**p- value**
Number of cases	63	206	-
Age *(in years)	44.7 (40.2-50.9)	52.8 (48.7-56.8)	<0.001
Sex (males)	66.7%	62.6%	0.559
Duration (years)	8.1 (3.7-15.0)	6.0 (2.4-11.2)	**0.049**
UPDRS score			
UPDRS Part 1	16 (14-19)	16 (14-18)	0.978
UPDRS Part 2	19 (11-26)	14 (9-22)	0.315
UPDRS Part 3	25 (17-41)	23 (14-34)	0.588
UPDRS Part 4	4 (0-10)	3 (0-8)	0.180
Hoehn Yahr stage	2 (1-3)	2 (2-3)	0.871
NMSS score			
Autonomic	10 (5-23)	10 (5-18)	0.868
Sleep/fatigue	10 (4-18)	8 (4-16)	0.635
Mood/apathy	6 (1-17)	4(1-12)	0.832
Percept. /halluc.	0 (0-1)	0 (0-2)	0.986
Attention/memory	4 (1-12)	4 (1-6)	0.842
Total score	45 (26-74)	37 (23-70)	0.801
ADL score	80 (60-90)	80 (63-90)	0.095
PDQ8SI score	34.4 (25.0-57.8)	31.3 (15.6-46.9)	0.285

*** Age at registration, IQR = interquartile range UPDRS = Movement disorder society unified Parkinson's disease rating scale, NMSS = non motor symptoms scale, Percept. = perception, Halluc. = hallucinations, ADL = activities of daily living using Schwab and England scale, PDQ8SI = Parkinson's disease quality of life summary index.**

There were also no difference in either the activities of daily living (Figure 11.11) or the quality of life scores (Figure 11.12 and 11.13) in those with a positive family history of PD compared to those with sporadic PD i.e. no family history of PD (Table 11.5).

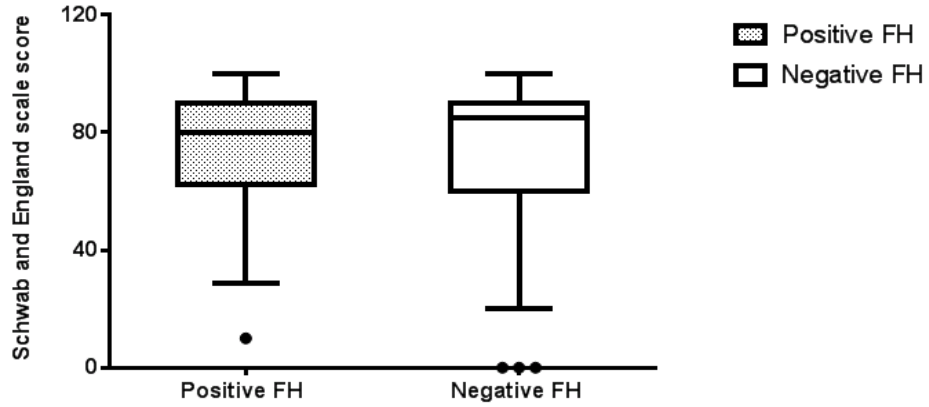

Figure 11.11 shows the Schwab and England scale score (on the y-axis) and the family history (FH) of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with familial PD compared to those with sporadic PD (p=0.371).

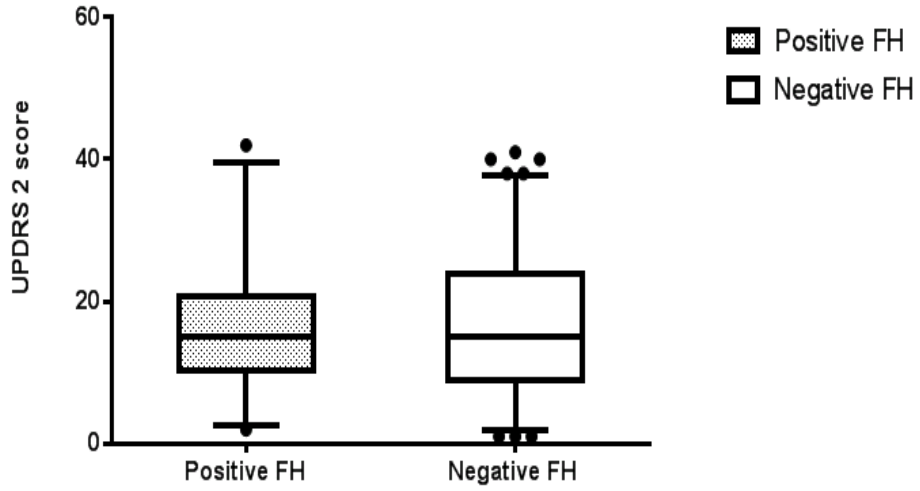

Figure 11.12 shows the movement disorder society unified Parkinson's disease part 2 (UPDRS 2) score (on the y-axis) and the family history (FH) of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with familial PD compared to those with sporadic PD (p=0.995).

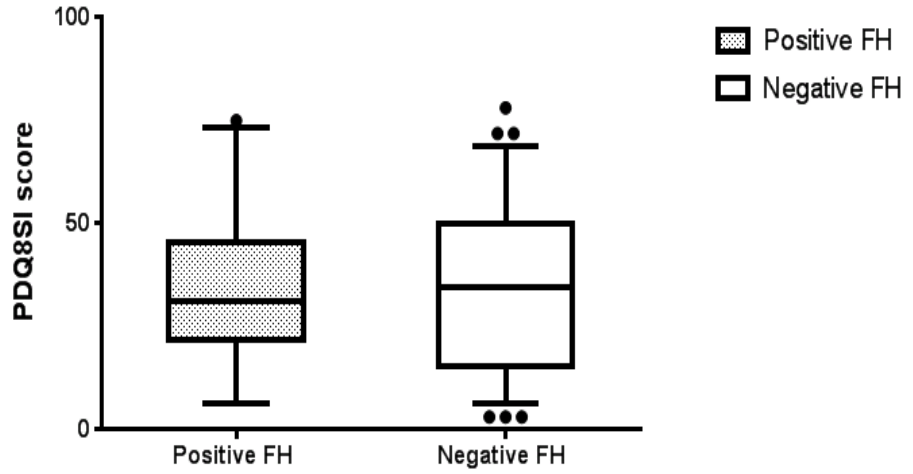

Figure 11.13 shows the Parkinson's disease quality of life summary index (PDQ8SI) score (on the y-axis) and the family history (FH) of PD (on the x-axis). The line in the centre of the boxes represent the medians, the ends of the whiskers 2 standard deviations on either side and outliers are represented by dark circles. No statistically significant differences were found between those with familial PD compared to those with sporadic PD (p=0.584).

11.5 Variation in variation in the activities of daily living (ADL), quality of life (QoL) scores and determinants of QoL in patients (n=269) classified by family history of PD.

Variable/ Scale	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p- value**
Number of cases	63	206	-
Age* (years)	52.3 (47.4-56.8)	51.0 (46.9-55.7)	0.296
Sex (males)	65.1%	63.1%	0.776
Duration (years)	6.9 (3.5-12.3)	6.7 (2.3-11.3)	0.239
UPDRS score			
UPDRS Part 1	16 (15-18)	16 (14-19)	0.998
UPDRS Part 2	15 (11-21)	15 (9-24)	0.995
UPDRS Part 3	25 (16-37)	24 (15-35)	0.671
UPDRS Part 4	5 (1-8)	3 (0-8)	0.112
Hoehn Yahr stage	2 (1-2)	2 (1-3)	0.711
NMSS score			
Autonomic	10 (4-18)	10 (5-19)	0.786
Sleep/fatigue	9 (5-17)	9 (4-16)	0.335
Mood/apathy	4 (1-12)	4 (1-13)	0.907
Percept. /halluc.	0 (0-2)	0 (0-2)	0.913
Attention/memory	3 (2-6)	4 (1-8)	0.752
Total score	41 (26-70)	39 (23-73)	0.831
ADL score	80 (65-90)	90 (60-90)	0.371
PDQ8SI score	32.8 (21.9-44.5)	34.4 (15.6-50.0)	0.584

FH = family history, * Age at registration, IQR = interquartile range. UPDRS = Movement disorder society unified Parkinson's disease rating scale, NMSS = non motorsymptoms scale, PDQ8SI = Parkinson's disease quality of life summary index, ADL = activities of daily living using Schwab and England scale.

11.5 Discussion

There is a complex mix of motor, non-motor, cognitive and neuropsychiatric symptoms that impact on the quality of life in patients with PD besides the social and personal circumstances of individual patients.

Besides its utility in clinical research, measuring or recording QoL scores in the health records of patients assumes importance as the overall aim of treatment in PD should be to minimize the negative impact of the disease on functioning in an attempt to maintain or improve the quality of life of these patients. There are several questionnaires to measure QoL, some universal that can be used for several diseases, others specific for one disease [422]. In our study we have employed a specific questionnaire: the Parkinson`s Disease Questionnaire 8 items (PDQ-8).

The PDQ-8 scale is an abbreviated version and derived from the Parkinson`s Disease Questionnaire 39 items (PDQ-39) scale [426]. The PDQ-8 has eight items (questions) measuring the physical and psychosocial impact of the disease. Each item represents one domain corresponding to the PDQ-9 scale. These domains are mobility, activities of daily living, emotional well-being, stigma of the disease (‘stigma’), social support, cognitive impairment (‘cognitions’), communication and bodily discomfort. The respondent (patient or carer) has to choose one of five possible answers: never (score 0), occasionally (score 1), sometimes (score 2), often (score 3) or always (score 4). The final score is obtained by adding the scores obtained from all 8 items. The range of scores on this scale is 0-32. In order to covert the score to a percentage, a summary index score based on answers to all the 8 questions - the PDQ-8 Summary Index (PDQ-8 SI) - can be derived as explained in the methods section [130].

A previous systematic review to compare and contrast the disease specific instruments to measure QoL in PD suggested that although the selection of an instrument partly depends on the goal of the study, in many situations the PDQ-39, of which PDQ-8 is a shortened form, is probably the most appropriate HRQoL instrument in PD based on the clinimetric properties and test-retest reliability of the scale [427].

The influence of the motor subtype on quality of life scores has been reported. Patients with the PIGD motor subtype reported worse QoL, compared with those with TDP [361]. This may be due the fact that patients with the PIGD subtype rate their mobility, ability to perfrom activities of daily living and communication worse than those with TDPD patients [428]. However, these reported differences need to be interpreted in the light of other deteminants of QoL such as disease duration and stage. Our results showed no differences in the quality of life scores ($p=0.796$) between the 3 motor subtypes having adjusted for differences between age, disease duration, UPDRS3, HY stages and ADL scores.

Gender based differences in the quality of life scores in PD has also been investigated. Females had lower quality of life scores [429] but paradoxically after DBS surgery [430] females emerge with higher quality of life scores, the suggestion being women have better coping strategies in the aftermath of surgery. Our results showed no differences in the quality of life scores between the two genders ($p=0.213$)

A previous study reported that quality of life scores were higher in those with younger onset (<55 years) compared to those with older onset (>55 years) of PD. Our results, however, showed no differences in the quality of life scores between those with onset of PD aged less than (or equal to) 40 years and those after the onset of 40 years in the EOPD cohort ($p=0.285$) In a study of familial PD (n= 30) versus sporadic PD (n=104) no differences were found in the quality of life scores between the 2 groups [323]. Our results replicated those results and showed no differences in the quality of life scores between those with a positive family history of PD compared to those with no family history of PD ($p=0.584$).

The variation in the prevalence of motor (examined in Chapter 5), non-motor (examined in Chapter 7) and neuropsychiatric symptoms like depression, apathy and impulsivity, (examined in Chapter 10), can explain part of the variation in activities of daily living and quality of life scores [431]. Even the place where care is provided to patients can be a contributor to the quality of life in PD suggesting that best practices in managing patients to improve their quality of lives may vary [432].

In summary, besides details obtained from the physical examination for assessment of the motor severity and a questionnaire survey to detect non-motor symptoms, quality of life

scoring tools can provide valuable insight into the health burden of PD and contribute to a more comprehensive picture of the total disease impact [433].

11.6 Conclusion

The variation in the quality of life scores are influenced by several factors including a mix of motor and non-motor symptoms however after making adjustments for co-variates such as age, disease duration, UPDRS3, HY stages and ADL scores, the differences in quality of life scores in a pre-selected, nearly homogeneous group of patients, the differences in the quality of life scores can even out. Adequate management of all these aspects of PD that impact on the quality of life require holistic care from clinicians and carers.

Chapter 12. Genetic influences causing variation in early onset Parkinson's disease

12.1 Objectives

The objectives of this chapter are to analyse the prevalence of genetic cases in early onset Parkinson's disease and the influence of genetic factors causing variation in the clinical phenotype.

12.2 Introduction

Identifying factors influencing phenotypic heterogeneity in PD is crucial for understanding variability in disease characteristics. Age, disease duration and gender are only the most basic epidemiological characteristics, that can influence the variation in the clinical expression of PD.[434] In the last decade and a half there has been substantial progress in our understanding of the genetics of PD. Highly-penetrant mutations in several genes (*SNCA, LRRK2, VPS35, Parkin, PINK1,* and *DJ-1*) can cause rare monogenic forms of the disease. Less penetrant mutations in the *LRRK2* and the *GBA* gene are strong risk factors for PD, and are especially prevalent in some ethnic groups. More recently, common variants of small effect size, modulating the risk for PD, have been identified by genome-wide association studies in more than 20 chromosomal loci [435]. Further details of how these mutations lead to alterations in the normal biochemical pathways and machinery of the cell that results in cellular damage leading to a parkinsonian phenotype are just emerging. As a corollary of their disease causing potential, mutations in these genes and single nucleotide polymorphisms at several other genetic loci together with epigenetic phenomena, contribute to the variation or heterogeneity in the phenotypic expression of PD. A summary of the genetic loci associated with PD are tabulated below.

Table 12.1 PARK loci linked with the Parkinson's disease phenotype.

Locus	Gene	Chromosome	Protein product	Inheritance	Reference
PARK1	*SNCA*	4q21.3-q22	Alpha-synuclein	AD	[6]
PARK2	*Parkin*	6q25.2-q27	Parkin	AR	[300]
PARK3	*NA*	2p13	NA	AD	[436]
PARK4	*SNCA*	4q21.3-q22	Alpha-synuclein	AD	[437]
PARK 5	*UCHLI-1*	4p13	Ubiquitin carboxyl-terminal esterase L1	AD	[438]
PARK6	*PINK1*	1p36.12	PTEN induced kinase 1	AR	[301]
PARK7	*DJ1*	1p36.23	DJ1	AR	[439]
PARK8	*LRRK2*	12q12	Leucine rich repeat kinase 2	AD	[440]
PARK9	*ATP13A2*	1p36	ATPase 13 A2	AR	[441]
PARK10	*Sus. loc.*	1p32	NA	NA	[442]
PARK11	*GIGYF2*	2q36-q37	GRB10 interacting GYF protein 2	AD	[443]
PARK12	NA	Xq21-q25	NA	X-linked	[444]
PARK13	*HTRA2*	2p13.1	HtrA serine peptidase 2	AD	[445]
PARK14	*PLA2G6*	22q13.1	Phospholipase A2	AR	[446]
PARK15	*FBX07*	22q12.3	F-box protein 7	AR	[447]
PARK16	*Sus. loc.*	1q32	NA	NA	[448]
PARK17	*VPS35*	16q12	Vacuolar protein sorting 35	AD	[449]
PARK18	*EIF4G*	3q27.1	Eukaryotic translation IF 4 gamma	AD	[450]
PARK19	*DNAJC6*	1p31.3	Auxilin	AR	[451]

AD = autosomal dominant, AR = autosomal recessive, NA = not available, *Sus. loc* = Susceptibility locus

Mutations in *GIGYF2* [443] and HTRA 2 [445] were found in PD cases, but subsequent studies have found such mutations in controls as well.[452, 453] .

Genetic testing for early onset Parkinson's disease (EOPD) is now offered by several centres in the United Kingdom. In Scotland as part of the services offered to NHS patients, Molecular Genetic Testing Laboratory at Ninewells hospital in Dundee offers a screen for *Parkin* copy number variation, direct sequencing of coding exons of *Parkin* and targeted sequencing of the most common *LRRK2* gene mutation in the northern European population. It is important to contrast what is offered as part of the standard NHS testing protocol in order to understand what PRoBaND seeks to determine in a research set-up.

12.3 Methods

Patients attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data at baseline (visit 0). Data required for the description of motor and non-motor symptoms using appropriate validated questionnaires including MDS-UPDRS [454], modified HY staging [455], NMSS [352], Schwab and England scale [424] , Leeds SAD [413], Leeds SAA [413], MOCA test [391], UPSIT [456], GCSI [351], PDSS[356] , ESS [355] , RBD [357] (as described in the earlier chapters) and medication requirements in LEU [333, 334] were recorded at study visit 1.

Genomic DNA was isolated from peripheral blood samples that were collected from patients at the baseline visit (visit 0) and shipped to Cardiff using standard protocols. In Cardiff, whole-genome DNA amplification was performed and then used for mutational screening by direct sequencing in *Parkin* and *GBA*. *LRRK2* genotyping for the *G2019S* point mutation using a 'Kompetitive' alelle specific polymerase chain reaction (KASP) assay (LGC Genomic solutions). Copy dosage analysis of *Parkin* was performed by multiplex ligation-dependent probe amplification (MLPA) on the genomic DNA obtained from blood samples using MLPA kits (obtained from MRC Holland) according to the manufacturer's protocol.

To analyse for the variation in demographic characteristics, motor, non-motor and cognitive descriptors all cases with EOPD were classified into gene test positive 'cases' and gene test negative 'controls' (comparator group) . Further analysis of the gene test positive cases was performed by classifying these cases into subgroups defined according to the genes that contained the pathogenic mutations i.e. *Parkin*, *GBA* and *LRRK2*.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Independent sample t-tests were used for inter-group comparisons of normally distributed continuous numerical data and Mann Whitney U test of continuous numerical data that was not normally distributed. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

In generalised linear models, based on ANCOVA, having adjusted for covariates (age , disease duration), a comparison was done between the two factors (independent variables) i.e. "cases" (gene test positive) and "controls" (gene test negative) with respect to all dependent variables (MDS-UPDRS scores). Additionally, when comparing MDS-UPDRS part 3 and MDS-UPDRS part 4 scores (dependent variables) daily anti-parkinsonian medication requirements in levodopa equivalent units were also used as a covariate. Logarithmic transformations (base 10) of numerical data were used where tests of normality (Shapiro Wilk) failed.

Finally binary logistic regression analysis was used to determine whether age of onset of PD in the EOPD cohort (less than 40 years versus greater than 40 years) had any association to the genes causing PD, gender, ethnicity or family history of PD.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (*version 23 for Windows*; IL, USA).

12.4 Results

178 patients with EOPD had genetic tests done for *Parkin*, *GBA* and *LRRK2* mutations. 18 tested positive for mutations in one of these 3 genes. 16 of these 18 patients had all questionnaires completed for the data analysis and are included here. 160 cases with EOPD tested negative for these genes. 134 of these had all questionnaires complete, for comparing their phenotypic characteristics with the gene test positive "cases", and are included in the "control" group (Figure 12.1).

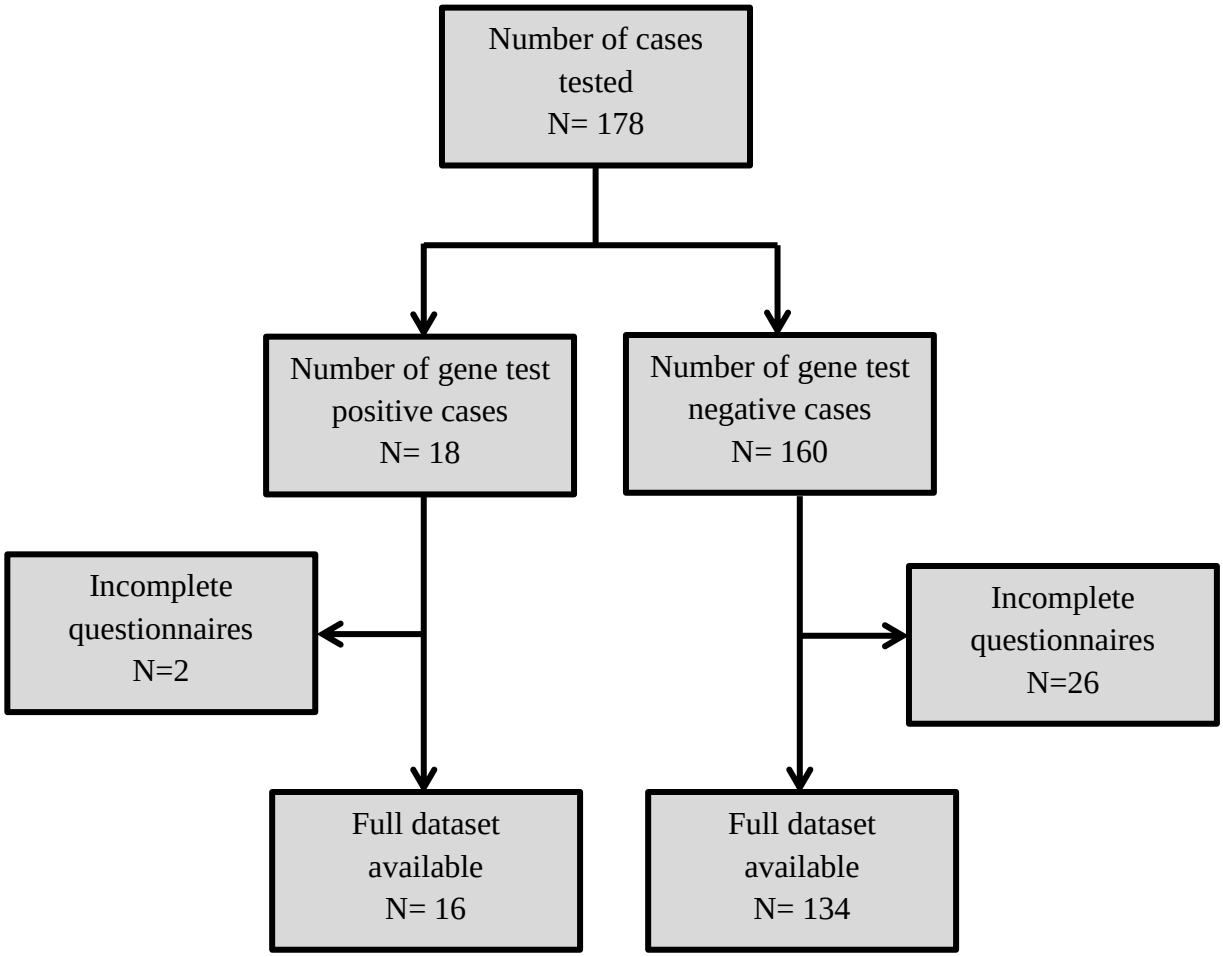

Figure 12.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The individual gene test results for the 16 cases and their demographic details are presented in Table 12.2.

Table 12.2 Demographics, motor disability and medication requirements of mutation carriers in the EOPD cohort from the PRoBaND study.

	Gene	**Mutation**	**Age** *	**Gender**	**HY**	**UPDRS3**	**LEDD**(mg/d)	**FH**
1.	*Parkin*	Ex 4 hz del / R275W	20.1 y	Male	3.0	32	2262	-ve
2.	*Parkin*	P113Xfs / R275W	40.0 y	Male	2.0	60	800	-ve
3.	*GBA*	N409S	48.2 y	Male	1.5	14	280	-ve
4.	*GBA*	D419A	38.7 y	Male	2.0	15	1320	+ve
5.	*GBA*	R502C	43.7 y	Male	2.0	20	925	-ve
6.	*GBA*	N409S	30.5 y	Female	1.0	9	1036	+ve
7.	*GBA*	D419A	45.7 y	Male	2.0	34	520	+ve
8.	*GBA*	P305Xfs	40.5 y	Male	2.5	28	1245	-ve
9.	*GBA*	L483P / A495P	42.0 y	Female	1.5	17	833	+ve
10.	*GBA*	L483P	32.9 y	Female	4.0	66	660	-ve
11.	*GBA*	L483P	43.4 y	Male	2.5	12	620	-ve
12.	*LRRK2*	G2019S	41.5 y	Male	2.0	30	1011	+ve
13.	*LRRK2*	G2019S	40.5 y	Male	1.5	22	2840	-ve
14.	*LRRK2*	G2019S	37.1 y	Male	1.5	11	1164	+ve
15.	*LRRK2*	G2019S	47.9 y	Male	1.0	25	100	+ve
16.	*LRRK2*	G2019S	44.0 y	Male	2.5	66	314	-ve

*** Age at registration in years (y), HY = Hoehn Yahr stage, UPDRS3 = modified unified Parkinson's disease rating scale Part 3, LEDD = Levodopa equivalent daily dose, FH = family history, +ve = positive, -ve = negative, Ex = exon, hz = heterozygous, del = deletion**

The demographics of the gene test positive patients (n=16), classified by gene of interest i.e. *Parkin*, *GBA* and *LRRK2 compared* to the gene test negative patients (n=134) are shown in Table 12.3.

Table 12.3 Demographics of 'cases' classified by gene mutation versus "controls"

Variable	***LRRK2*** median (IQR)	***GBA*** median (IQR)	***Parkin*** median (IQR)	**"Controls"** (Gene- test negative) median (IQR)
Number	5	9	2	134
Age* (years)	52.1 (50.5-53.3)	50.2 (47.1-55.5)	52.7 (50.7-54.8)	53.4 (48.6-57.4)
Duration (years)	10.0 (6.2-15.0)	7.3 (4.8-16.4)	22.7 (19.8-25.6)	8.4 (5.4-12.4)
Sex (males)	100 %	66.7 %	100 %	66.2 %
Ethnicity				
Caucasian	100 %	88.9 %	100 %	95.5 %
Asian	-	11.1 %	-	3.0 %
African	-	-	-	-
Caribbean	-	-	-	0.8%
Positive FH	60.0 %	44.4 %	-	25.9 %
ADL‡	80 (60-90)	90 (80-90)	55 (42.5-67.5)	80 (60-90)

*** Age at registration, *LRRK2* = Leucine rich repeat kinase 2 gene test positive, *GBA* = Glucocerebrosidase gene test positive, *Parkin* = Parkin gene test positive, FH= family history ‡ADL=activities of daily living score is based on Schwab and England scale**

The disease duration was longest in the *Parkin* patients compared to the other groups (p=0.037) and keeping in with the autosomal recessive nature of inheritance of this gene mutation, there was no family history of PD in the preceding or succeeding generations.

The variation in mentation, behaviour and mood symptoms, recorded using MDS- UPDRS scale Part 1 (figure 12.2), activities of daily living, recorded using MDS- UPDRS scale Part 2

(figure 12.3), motor features recorded using MDS- UPDRS scale Part 3 (figure 12.4), and complications of PD treatment, recorded using MDS- UPDRS scale Part 4 (figure 12.5) are shown in Table 12.4 by classifying "cases" (gene test positive) according to gene test abnormality versus "controls" (gene test negative).

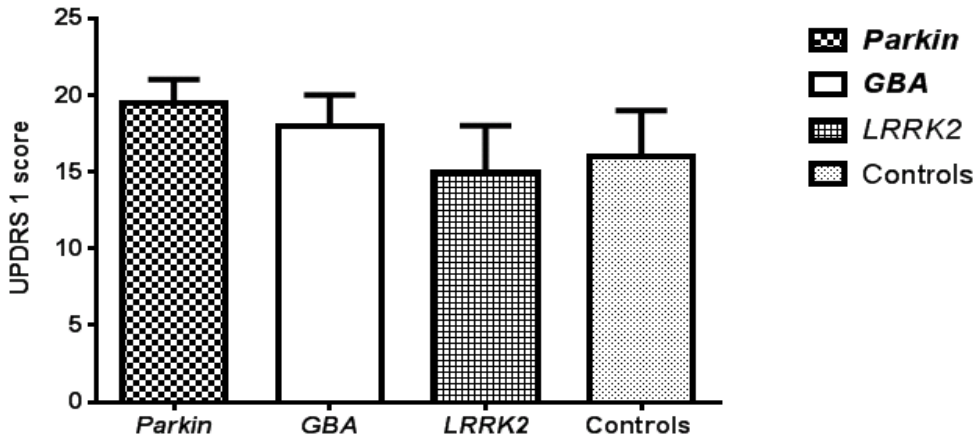

Figure 12.2 shows the movement disorder society unified Parkinson's disease rating scale (MDS-UPDRS) Part 1 scores on the y-axis and the two groups EOPD subjects classified by gene test mutation and "controls" (gene test negative) on the x-axis. The top of the bar column graphs represents the median and the whiskers represent the inter-quartile range (bottom whisker is hidden within the boxes). Due to very small sample sizes (n=2) in some groups, no inferences have been made about inter-group differences.

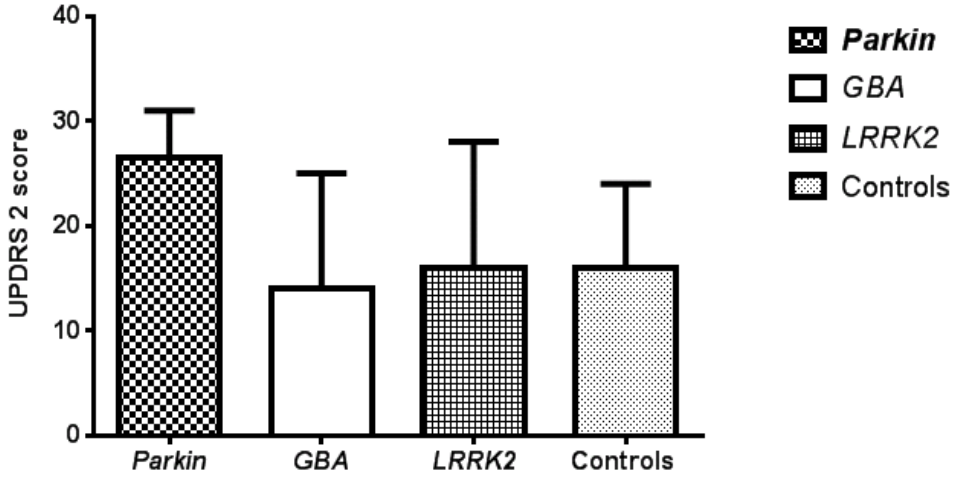

Figure 12.3 shows the movement disorder society unified Parkinson's disease rating scale (MDS-UPDRS) Part 2 scores on the y-axis and the two groups EOPD subjects classified by gene test mutation and "controls" (gene test negative) on the x-axis. The top of the bar column graphs represents the median and the whiskers represent the inter-quartile range (bottom whisker is hidden within the boxes). Due to very small sample sizes (n=2) in some groups, no inferences have been made about inter-group differences.

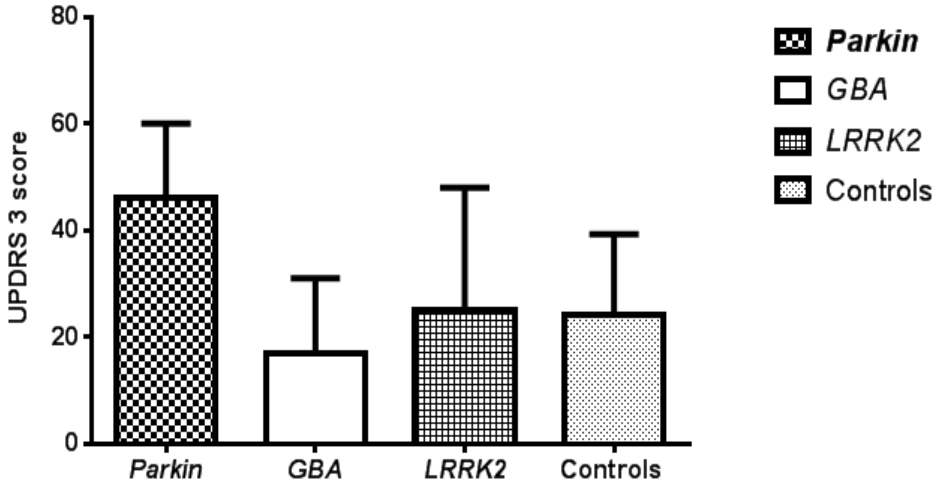

Figure 12.4 shows the movement disorder society unified Parkinson's disease rating scale (MDS-UPDRS) Part 3 scores on the y-axis and the two groups EOPD subjects classified by gene test mutation and "controls" (gene test negative) on the x-axis. The top of the bar column graphs represents the median and the whiskers represent the inter-quartile range (bottom whisker is hidden within the boxes). Due to very small sample sizes (n=2) in some groups, no inferences have been made about inter-group differences.

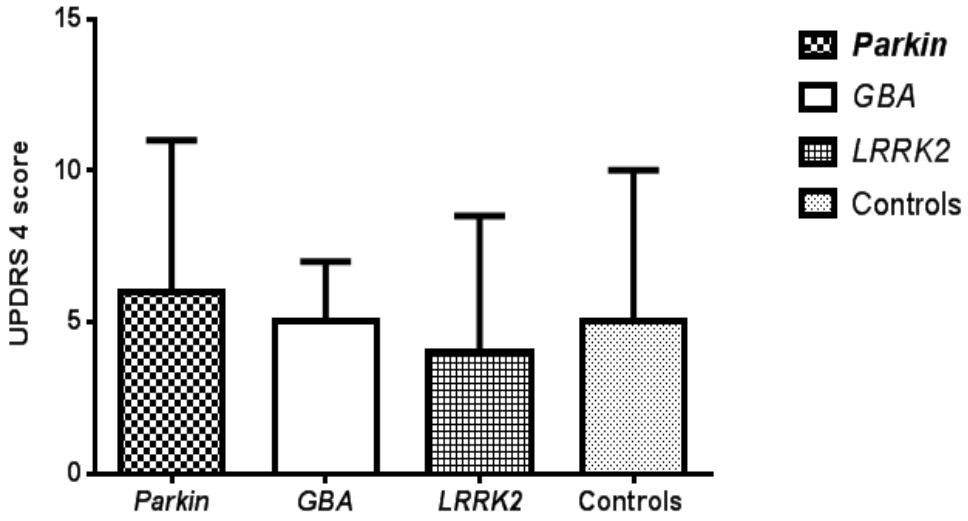

Figure 12.5 shows the movement disorder society unified Parkinson's disease rating scale (MDS-UPDRS) Part 4 scores on the y-axis and the EOPD subjects classified by gene test mutation and "controls" (gene test negative) on the x-axis. The top of the bar column graphs represents the median and the whiskers represent the inter-quartile range (bottom whisker is hidden within the boxes). Due to very small sample sizes (n=2) in some groups, no inferences have been made about inter-group differences.

There were no significant differences in the MDS-UPDRS total and sub-scores between the pooled data for the gene test positive cases and gene test negative cases when adjustments were made for age, disease duration and medication requirements in the statistical model for any baseline differences in these variables (not shown in tables).

Table 12.4 MDS-UPDRS scores, Hoehn Yahr stages and medication requirements in "cases" classified by genotype versus "controls".

Variable	***LRRK2*** median (IQR)	***GBA*** median (IQR)	***Parkin*** median (IQR)	**"Controls"** (Gene- test negative) median(IQR)
Number	5	9	2	134
UPDRS 1	15 (14-15)	18 (17-20)	19 (18-20)	16 (14-19)
UPDRS 2	16 (11-27)	14 (13-24)	26 (24-28)	16 (10-24)
UPDRS 3	25 (22-30)	17 (14-28)	46 (39-53)	24 (17-39)
UPDRS 4	4 (1-8)	5 (4-6)	6 (3-8)	5 (1-10)
UPDRS total	70 (43-71)	61 (54-74)	98 (96-99)	65 (47-84)
HY stage	1.5 (1.5-2.0)	2.0 (1.5-2.5)	2.5 (2.25-2.75)	2.0 (1.5-2.5)
LEU(mg/day)	1011 (314-1164)	833 (620-1036)	1530 (1165-1896)	798 (524-1023)

MDS-UPDRS = Movement disorder society unified Parkinson's disease rating scale, *LRRK2* = Leucine rich repeat kinase 2 gene test positive, *GBA* = Glucocerebrosidase gene test positive, *Parkin* = Parkin gene test positive, UPDRS = Movement Disorder Society Unified Parkinson's disease rating scale, UPDRS1 = UPDRS Part 1 score, UPDRS 2 = MDS-UPDRS Part 2 score, UPDRS 3 = MDS-UPDRS Part 3 score, UPDRS 4 = MDS-UPDRS Part 4 score, HY = Hoehn Yahr stage, LEU= Levodopa equivalent dose, HY = Hoehn Yahr stage, LEU= Levodopa equivalent unit dose

The number of cases in some groups were so small (n=2) that the results of non-parametric statistical tests such as the chi-square test for categorical data comparing 3 groups would not be valid. The power of any test to detect any significant differences between groups is handicapped when the numbers are small. This was, therefore, not attempted in Tables 12.3, 12.4 and 12.5.

A detailed analysis of the non-motor symptoms of dysfunction sleep such as excessive and REM sleep behaviour disorder; neuropsychiatric symptoms such as anxiety and depression; autonomic features such as constipation and bladder dysfunction; cognitive problems including dementia recorded using appropriate and validated scales, described in the methods section, are shown in Table 12.5 by classifying patients into ''cases'' (gene test positive) according to gene test abnormality versus ''controls'' (gene test negative).

Table 12.5 Non-motor features in "cases" classified by genotype versus "controls".

Variable	***LRRK2***	***GBA***	***Parkin***	**"Controls"** (Gene- test negative)
Number	5	9	2	134
EDS	60 %	55.6 %	100 %	49.7 %
Disrupted sleep	60 %	22.2 %	100 %	35.7 %
RBD	100 %	55.6 %	50 %	53.8 %
Anxiety*	40.0 %	44.4 %	50.0 %	46.9 %
Depression**	20.0 %	55.6 %	50.0 %	43.4 %
Anosmia£	M: 60 % F: NC	M: 50 % F: 50 %	M: 0 % F: NC	M: 50.6 % F: 15.0 %
Constipation	20.0 %	44.4 %	50.0 %	53.0 %
Bladder dysfunction†	100 %	100 %	100 %	93.3 %
Cognitive impairment§	100 %	88.9 %	50 %	83.8 %
Dementia ‡	20 %	11.1 %	0 %	9.7 %

***LRRK2* = Leucine rich repeat kinase 2 gene test positive, *GBA* = Glucocerebrosidase gene test positive, *Parkin* = Parkin gene test positive, EDS = Excessive daytime sleepiness based on an Epworth Sleep Scale score >=9 , Disrupted sleep based on a Parkinson's disease sleep score (PDSS) >= 100, *based on Leeds scale for self-assessment of anxiety (SAA) score >= 7, **based on Leeds for self-assessment of depression (SADS) score >=7, † Bladder dysfunction= Bladder dysfunction= difficulty retaining urine, feeling bladder not completely empty, weak stream, pis en deux or nocturia, § based on Montreal cognitive assessment (MOCA) test score <26 , ‡ based on a MOCA test score < 20, £ based on University of Pennsylvania Smell Identification test (UPSIT) score <19 in males and females, NC = no cases.**

Binary logistic regression analysis showed age of onset of PD in the EOPD cohort (less than 40 years versus greater than 40 years) was not related to the genes causing PD (p=0.948), gender (p=0.420), ethnicity (p=0.894) or family history of PD (p=0.234).

12.5 Discussion

The detailed molecular mechanisms of the pathogenesis leading to neuronal death in PD remain largely unknown; however, it is becoming increasingly clear that genetic factors have a role to play in the inheritance patterns and the underlying neuronal degeneration in the brain of this complex trait. In the past 15 years, the genetic basis of rare forms of PD with Mendelian inheritance, which represent less than 10% of total cases, has been investigated. More than 16 loci, identified through linkage analysis or genome wide association studies (GWAS), and eight validated genes have been identified so far (Table 12.1). Several studies have shed light on the influence of these genes not only in familial but also in sporadic forms of PD [302].

The prevalence of genetic cases of PD overall is reported to be 5-10% [457]. Our study showed a prevalence of 10.1% of genetic cases of PD in the EOPD cohort randomly tested for mutations in 3 genes *Parkin, GBA* and *LRRK2*. This is likely to under report the number of cases with a genetic basis to PD given that there are other genes such as *PINK1*, *DJ1* and *SNCA* that we didn't test (Table 12.1) in this phase of our study but have been tested in other studies [458-460] . In our study only 2 genes were sequenced (*Parkin* and *GBA*) and only targeted mutational analysis was performed on *LRRK2* (*G2019S*) at this stage based on the frequencies of these genes being implicated in the general PD population [166, 435], the other genes being less prevalent in the overall population, while recognising the fact that certain gene mutations such as *PINK1* may be more common in certain ethnic backgrounds [458].

The most common mutation in our study was in *GBA* (5.6%) and the least common was in *Parkin* (1.7%) with *LRRK2* mutations somewhere in between (2.8%). *GBA* is the most common cause for genetic cases of PD in the British population and has been reported before [166] but we believe this is the first time this has been replicated in an EOPD cohort. This may have implications for genetic testing offered locally for NHS patients. The east of Scotland regional molecular genetics service based in the molecular genetics department at Ninewells Hospital, Dundee, has been offering, a free at the point of care, service to NHS patients in the whole of Scotland. Over the years the testing that has been offered is restricted

to *Parkin* whole gene sequencing and targeted mutation analysis of *G2019S* (p.Gly2019Ser) mutation in the *LRRK2* gene supplemented by a gene dosage analysis of the *Parkin* gene using MPLA kits. Restricting the genetic testing to *Parkin* and *LRRK2* in the EOPD misses the largest segment of patients with genetic PD i.e. *GBA* mutation carriers and should be included as this has implications for genetic counselling of families given this is an autosomal dominant gene with 1:2 chance of passing the mutated gene to the offspring. The importance of *GBA* testing is not overstated given *GBA* mutations can influence the natural history of Parkinson's disease in a community-based incident cohort on longitudinal follow up. The hazard ratio for progression both to dementia and Hoehn and Yahr stage 3 were significantly greater in *GBA* mutation carriers than those who were wild-type homozygotes [461]. This is reported to be the first time a genetic locus has been shown to influence motor progression in PD and therefore a potential prognostic marker but needs to be confirmed in other studies such as PRoBaND over time.

Few large scale studies have ever been performed in homogeneous populations to explore the contribution of known PD-causing genes in patients with EOPD. In a previous study of 38 patients with PD in southern Africa (18% with positive familial history of PD) with an average onset age 54.9 ± 12.2 years, all *SNCA* exons and *LRRK2* exons 29 to 48 were sequenced in every patient. In those patients with a family history of PD and those with age at onset <55 years (n = 22) the whole *LRRK2* coding region was sequenced (51 exons). In the patients with onset <50 years (n = 12), all *Parkin, PINK1, and DJ-1* exons were sequenced, and dosage analysis of *Parkin, PINK1, DJ-1, LRRK2*, and *SNCA* was performed. The *LRRK2* p.Gly2019Ser mutation was not detected. A novel *LRRK2* missense variant (p.Ala1464Gly) of possible pathogenic role was found in one case [459]. In our study however p.Gly2019Ser mutation was the second most common mutation detected (5/18) in the EOPD cohort. *G2019S* (p.G2019S) in *LRRK2* is the most common mutation in this gene [462] but screening for only a single mutation (targeted mutation analysis) suggests a great disparity in the proportion of patients with *LRRK2* mutations amongst different groups (42% in Tunisian families and 2.6% in U.S. families [463]. In the southern African study no case with this mutation was found, in our study this accounted for 2.8% of cases. While it is completely plausible that different mutations segregate in different populations due to a founder effect, the overall reported frequency of mutations in this gene will understandably be deflated unless all the exons are sequenced as several other mutations in *LRRK2* can cause PD [462].

This may be one of the weaknesses of our study as well at this stage given only targeted mutational analysis has been performed for *G2019S LRRK2* mutation rather than sequencing the whole gene.

The study recruiting patients in sub-Saharan Africa reported two heterozygous, likely disease-causing deletions of *Parkin* (exon 2 and exon 4) in the early-onset cases [459]. In our study one patient had a hemizygous deletion on exon 4 along with R275W mutation, both previously reported. *Parkin* mutations (3/18) were the least common cause for genetic cases of PD in the PRoBaND EOPD cohort. In the southern African study no pathogenic mutations were detected in *SNCA, PINK1,* or *DJ-1,* another hint that some mutations segregate in certain ethnic groups. In fact no common disease-causing mutations were detected in the 5 genes tested, suggesting that further investigations in PD patients from different populations might unravel the role of additional, still unknown genes [459].

The methodology used in the southern African study and our study has important implications in the reporting of the genetic contributions to PD. Direct sequencing of genes for point mutations or targeted sequencing for point mutations would miss copy number variations (gene dosage). Genomic rearrangements account for a substantial number of the genetic cases of PD [460]. MLPA (multiplex ligation-dependent probe amplification), is an alternative technique, based on a multiplex polymerase chain reaction (PCR) method can detect abnormal copy numbers such as duplications and (partial) deletions. These techniques are therefore complimentary when screening genes for PD and studies reporting the use of only of the 2 techniques will under report the genetic cases of PD.

The second point to note is some studies have reported novel mutations that have later turned out to be non-pathogenic and considered variant polymorphisms, for example, the R275W variant in *Parkin* is still controversial because this mutation was found to occur as frequently in control subjects (3 of 192) as in patients (5 of 313) [464], unless a second mutation is also identified. In light of the above, in our study R275W missense mutation in combination with exon 4 hemizygous deletion (Case 1 in Table 12.2) and mutation P113Xfs (Case 2 in Table 12.2) can explain the disease state in both patients. In the PRoBaND EOPD cohort all mutations reported in the 3 genes sequenced have previously been reported except a novel P305Xfs mutation in *GBA* that has not been previously reported.

A systematic review and UK-based study of PARK2 (*Parkin*), PARK6 (*PINK1*), PARK7 (*DJ-1*) and PARK8 (*LRRK2*) in EOPD showed an increased likelihood of mutations in patients with lower age at onset, family history, or parental consanguinity [458]. Our study, however, showed that the age at onset (less than 40 years versus greater than 40 years) was not related to the genes causing PD ($p = 0.948$), gender ($p = 0.420$), ethnicity ($p = 0.894$) or family history of PD ($p = 0.234$) using binary logistic regression. The age of onset considered in that systematic review dichotomised patients into those with age of onset < 30 years versus age at onset > 30 years. Only 1 patient in our EOPD cohort who tested positive for any gene mutation had an age of onset < 30 years, all others had age at onset > 30 years, hence the rationale for considering the split between the 2 groups at 40 years to test this hypothesis.

In our study the results suggest it would clinically not be possible to distinguish gene test positive cases from gene test negative cases in the EOPD cohort based simply on the phenotype given that the differences in clinimetric measures (Tables 12.4) between the 2 groups, when the pooled data was analysed (not shown in tabulated form) for all those with gene test mutation positive status compared to those with gene test mutation status, did not show any significant differences after adjusting for the effects of age, disease duration and medication, except for *Parkin* mutation carriers. *Parkin* mutation carriers had an earlier onset and longer disease duration (Table 12.3) which has also been reported before [169]. The limitation placed by the small numbers of cases who tested positive for mutations in each PD causing gene that was analysed in this study when analysing differences between groups, to search for statistically significant differences, was recognised. This was therefore not attempted, in order to avoid drawing erroneous conclusions based on a dataset with small number of genetically proven PD cases, which could not be replicated by other research groups.

The implications, therefore, are that clinical phenotypes of gene mutation carriers overlap with those who test negative for the genes involved in PD, with the exception of *Parkin* carriers, to such an extent that it would not be possible predict genotype based on phenotype, however, that should not cloud the importance that gene mutation carrier status has on disease course and prognosis; therein lies the main utility of gene testing, in addition to its

relevance in inheritance risks and genetic counselling. However, genetic testing in PD can raise ethical issues as well as issues of informed consent if presymptomatic testing is performed and if this testing is offered to first degree relatives of patients with autosomal dominant PD genes.

12.6 Conclusion

Patients with EOPD should be offered genetic testing in order to further understand their disease, to enable them to make informed life decisions and with a more secure diagnosis they can be enrolled in trials for specific treatments such as *LRRK2* kinase inhibitors.

Chapter 13. Environmental influences causing variation in Parkinson's disease

13.1 Objective

The objective of this chapter is to analyse the variation in environmental risk factors in a cohort of patients with early onset Parkinson's disease.

13.2 Introduction

The link between PD and environmental influences grew after a mini-epidemic of cases exposed to the synthetic meperidine (pethidine in the United Kingdom) derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) emerged in the San Francisco Bay area in intravenous drug users [465]. MPTP induces parkinsonism in humans by selectively destroying nigrostriatal dopaminergic neurons [466]. This discovery not only helped establish animal models of PD [467] but also stimulated an interest in finding chemically similar molecules that could replicate the effects of MPTP on the substantia nigra. Numerous population based epidemiological surveys have looked at the possibility of one or more environmental exposures that could either increase or decrease the lifetime risk of developing PD.

13.3 Methods

Patients from the EOPD cohort of the PRoBaND study were the subject of analysis for the environmental influences in PD. They attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic

data at baseline (visit 0). They were asked to fill in a modified mini environmental risk questionnaire for Parkinson's disease patients' baseline (MERQ-PD-B) at study visit 2 as described in detail in Chapter 4.

In order to analyse for variation in environmental influences affecting PD phenotypic expression with respect to medication exposure , all cases with EOPD were classified in 4 ways: according to motor subtypes i.e. TDPD, PIGD and 'Mixed' , gender, age at onset of PD symptoms and heritability of the Parkinsonian trait as described in Chapter 4.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U test if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (*version 23 for Windows*; IL, USA).

13.4 Results

210 patients had completed all questionnaires relevant to environmental exposures analysis (Figure 13.1) and this is indicated in the relevant tables (Tables 13.1-13.4). Their demographic details have been presented in Table 4.2 in Chapter 4.

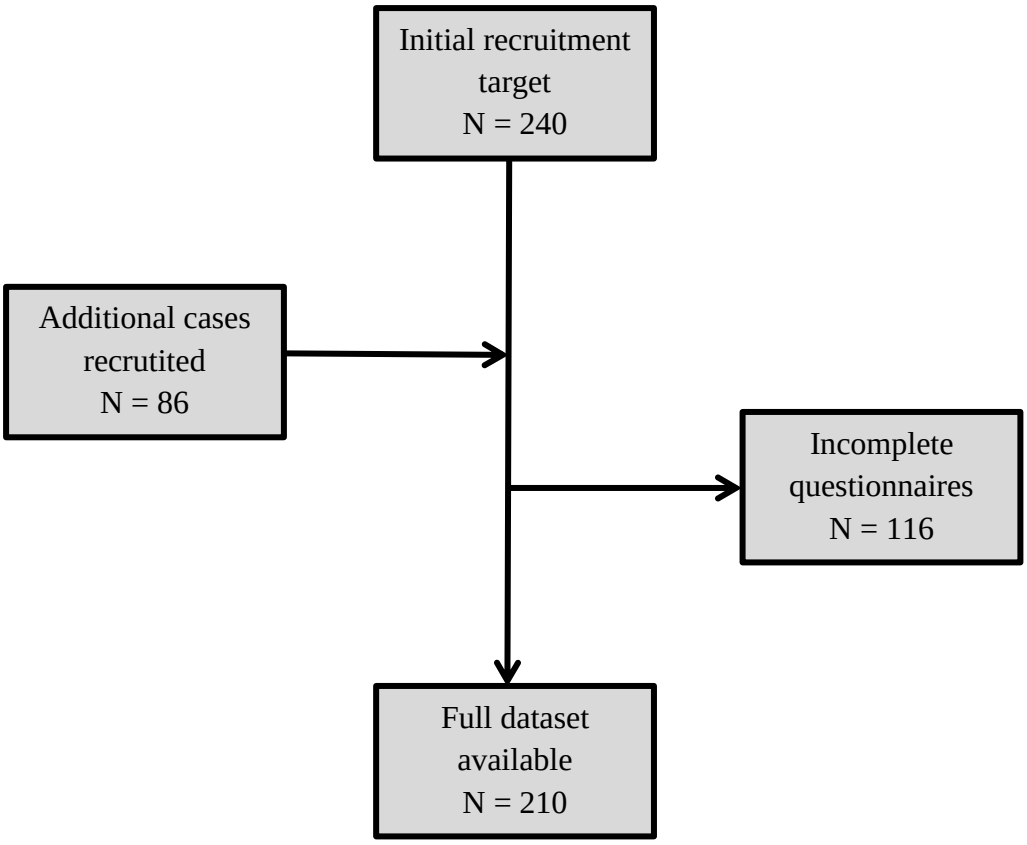

Figure 13.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in the exposure to environmental risk factors, classified by motor subtype, is presented in Table 13.1. There were no statistically significant differences in any of the environmental risk exposures that are considered to either decrease the risk of developing PD (Figure 13.2) or increase the risk of developing PD (Table 13.1) between the three motor subtypes.

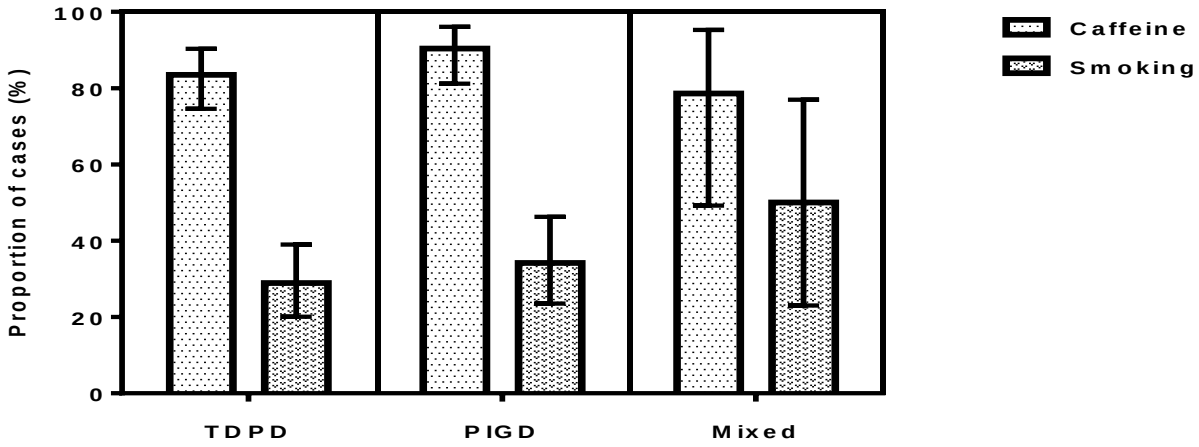

Figure 13.2 shows the proportion of patients who smoked or consumed caffeine in significant amounts (≥ 3 cups of coffee/tea per day) before the diagnosis of PD, as recorded on the MERQ-PD baseline questionnaire (on the y-axis) and their motor subtype (on the x-axis). There were no significant differences in the exposure to smoking (p=0.268) or caffeine (p=0.992, p=0.370) between the three motor subtypes.

Table 13.1 Variation in environmental exposures (as risk factors) in cases (n=210) from the EOPD cohort classified by motor subtype

Variable/ Exposure	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number	116	79	15	-
Age*(years)	51.2 (46.0-54.7)	55.0 (50.0-59.4)	51.2 (45.9-52.3)	**<0.001**
Sex (males)	65.5 %	59.5 %	60.0 %	0.675
Duration (years)	5.7 (2.9-10.1)	9.5 (5.4-15.1)	7.7 (3.9-11.2)	**<0.001**
Solvents †‡	21.6 %	27.4 %	14.3 %	0.480
Pesticides§	52.6 %	53.4 %	28.6 %	0.214
Heavy metals †‡	13.4 %	12.3 %	0 %	0.837¶
Smoking †	28.9 %	34.2 %	50.0 %	0.268
Caffeine intake †				
< 3 cups/ day	14.4 %	15.1 %	14.3 %	0.992
≥ 3 cups/ day	65.5 %	65.8 %	73.3 %	0.370

TDPD = tremor dominant Parkinson's disease, PIGD = postural instability gait difficulty, * age at registration, † before diagnosis, ‡ exposure for more than 6 months, § includes herbicides, insecticides, fungicides and fumigants, ¶ Fisher's exact test between TDPD and PIGD groups (chi-square not valid when comparing cells with values less than 5 therefore mixed subtype not used in the equation)

Classifying patients by gender showed no statistically significant differences in any of the environmental risk exposures that are considered to decrease the risk of developing PD (Table 13.2) but amongst risk factors that are reported to increase the risk of developing PD, exposure to solvents ($p<0.001$) and heavy metals were more commonly reported by males compared to females (Figure 13.3).

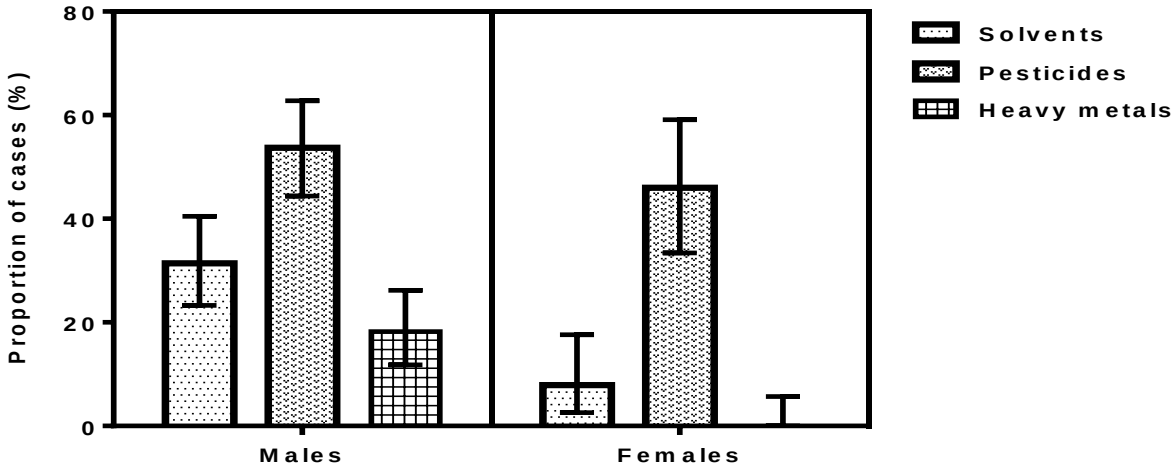

Figure 13.3 shows the proportion of patients who were exposed to solvents, pesticides or heavy metals before the diagnosis of PD, as recorded on the MERQ-PD baseline questionnaire (on the y-axis) and their gender (on the x-axis). Exposure to solvents ($p<0.001$) and heavy metals ($p<0.001$) were more commonly reported by males compared to females

Table 13.2 Variation in environmental exposures (as risk factors) in cases (n=210) from the EOPD cohort classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p- value**
Number of cases	132	78	-
Age*(years)	52.5 (47.4-56.7)	52.2 (47.4-56.5)	0.906
Disease duration (years)	7.7 (3.7-11.6)	6.7 (3.3-11.3)	0.362
Solvents †‡	31.4 %	7.9 %	**<0.001**
Pesticides§	53.7 %	46.0 %	0.332
Heavy metals †‡	18.2 %	0 %	**<0.001**
Smoking †	37.2 %	23.8 %	0.070
Caffeine intake †			
< 3 cups/ day	12.4 %	19.0 %	0.273
≥ 3 cups/ day	66.7 %	65.4 %	0.849

*** Age at registration, † before diagnosis, ‡ exposure for more than 6 months, § includes herbicides, insecticides, fungicides and fumigants.**

There were no statistically significant differences in any of the environmental risk exposures that are considered to either decrease the risk of developing PD or increase the risk of developing PD (Figure 13.4) between those diagnosed with PD aged less than (or equal to) 40 years and those diagnosed with PD after the age of 40 years (Table 13.3).

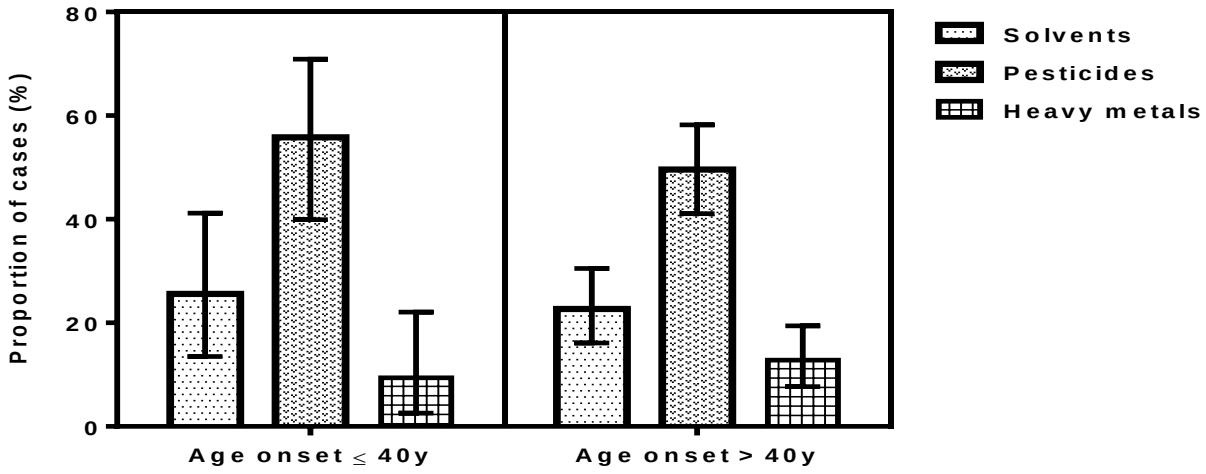

Figure 13.4 shows the proportion of patients who were exposed to solvents, pesticides or heavy metals before the diagnosis of PD, as recorded on the MERQ-PD baseline questionnaire (on the y-axis) and their age at onset of PD (on the x-axis). There were no significant differences in the exposure to solvents (p=0.685), pesticides (p=0.492) or heavy metals (p=0.788) between those with age at onset ≤ 40 years and those with age at onset > 40 years.

Table 13.3 Variation in environmental exposures (as risk factors) in cases (n=210) from the EOPD cohort classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40 yrs.** median (IQR)	**p-value**
Number of cases	50	160	-
Age* (years)	47.2 (41.6-51.9)	53.5 (49.7-57.0)	**<0.001**
Sex (males)	72.0 %	60.0 %	0.135
Disease duration (years)	8.4 (4.7-15.0)	7.2 (3.1-11.2)	**0.031**
Solvents †‡	25.6 %	22.7 %	0.685
Pesticides§	55.8 %	49.6 %	0.492
Heavy metals †‡	9.3 %	12.8 %	0.788
Smoking †	39.5 %	30.5 %	0.271
Caffeine intake †			
< 3 cups/ day	14.0 %	14.9 %	0.879
≥ 3 cups/ day	58.0 %	68.8 %	0.161

*** Age at registration, † before diagnosis, ‡ exposure for more than 6 months, § includes herbicides, insecticides, fungicides and fumigants.**

There were no statistically significant differences in any of the environmental risk exposures that are considered to either decrease the risk of developing PD or increase the risk of developing PD (Figure 13.5) between those with a positive family history of PD compared to those with a negative family history of PD (Table 13.4).

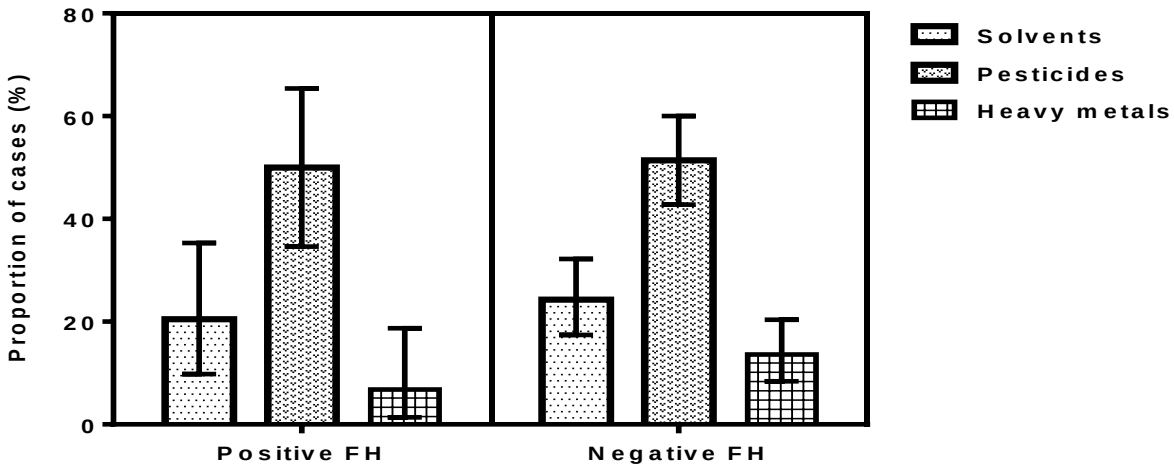

Figure 13.5 shows the proportion of patients who were exposed to solvents, pesticides or heavy metals before the diagnosis of PD, as recorded on the MERQ-PD baseline questionnaire (on the y-axis) and their family history (FH) of PD (on the x-axis).

Table 13.4 Variation in environmental exposures (as risk factors) in cases (n=210) from the EOPD cohort classified by family history of PD.

Variables	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	52	158	-
Age* (years)	52.0 (47.4-55.9)	52.7 (47.5-56.6)	0.586
Sex (males)	63.5 %	62.7 %	1.000
Disease duration (years)	6.0 (3.7-11.3)	7.6 (3.7-11.7)	0.602
Solvents †‡	20.5 %	24.3 %	0.686
Pesticides§	50.0 %	51.4 %	0.869
Heavy metals †‡	6.8 %	13.6 %	0.294
Smoking †	38.6 %	30.7 %	0.359
Caffeine intake †			
< 3 cups/ day	13.6 %	15.0 %	0.824
≥ 3 cups/ day	61.5 %	67.7 %	0.414

FH = family history, * age at registration, † before diagnosis, ‡ exposure for more than 6 months, § includes herbicides, insecticides, fungicides and fumigants.

13.5 Discussion

Environmental influences that have been linked to PD include pesticides [468], heavy metals [469] , solvents [470] , smoking [471] , coffee [472] , rural living [473] and well water [474]. Although some of the findings have not been replicated across all surveys but exposure to pesticides increasing the risk of PD while smoking and coffee consumption decreasing the risk seem to stand out even in meta-analyses [194].

The influence of gender *vis a vis* environmental risk factors such as coffee drinking has been reported in a meta-analysis of 8 case–control studies and 5 cohort studies on coffee drinking [475]. Interestingly, the two cohort studies in this meta-analysis that included only men (i.e., the Honolulu Heart Study and the Health Professionals' Follow-up Study) [472, 476] found a strong inverse linear relation between cups of coffee and risk of PD (pooled relative risk per three additional cups/day, 0.51; 95% confidence intervals, 0.31–0.83), whereas the cohort study that included only women (i.e., the Nurses' Health Study) [475] found a virtually null linear relation (relative risk per three additional cups/day, 1.00; 95% confidence intervals, 0.74–1.34). Our results didn't show any differences in coffee intake between the genders (Table 13.2). The only difference in exposure to any of the environmental risk factors was the prevalence of exposure to solvents which was reported more by males compared to females ($p<0.001$).

The influence of environmental factors on the age of onset of PD has also been investigated. In a study of 80 patients with LOPD (> 60 years), 69 EOPD (<40 years), and 149 age- and sex-matched control subjects, there were no significant differences in early life experiences or environmental exposures between the EOPD and LOPD patients [477]. Our results also showed no differences in the exposure to environmental agents considered in this study in between the two groups (Table 13.3).

The influence of environmental risk factors on the motor subtype of PD has been reported. In a study of 212 PD patients and 175 age- and gender-matched controls showed that the inverse association of smoking ($p=0.046$) and alcohol consumption ($p=0.07$) was only seen in PIGD cases and not in TDPD cases, which was similar to controls [478]. Our results, however, showed no significant differences in the exposure to environmental agents considered in this study in between the three groups (Table 13.1) suggesting that the motor subtypes of PD are not influenced by environmental risk factors, considered in this analysis, preceding the diagnosis of PD.

Those with a positive family history of PD, particularly across more than 2 generations, would very likely to point to a heritable trait and therefore genetic factors responsible for the aetiology , however, for those with sporadic PD one can hypothesize that there must be some environmental risk factor that is related to the disease process in question. Surprisingly there

was no difference in exposure to environmental agents considered in this study in between the two groups (Table 13.4).

We did not attempt to analyse whether certain environmental influences increase or decrease the chances of developing PD during a person's lifetime for that would require a much larger cohort of patients and controls to draw any definite conclusions, perhaps some of those answers can only be gained from meta-analyses of datasets from several large studies pooled together. In this study, our aim was only to find out whether certain environmental influences were more or less likely to be associated with certain subtypes of PD, and more specifically to analyse this issue within an EOPD cohort. On their own each of these environmental risk factors, that we analysed, can not be sufficient to explain all the heterogeneity of PD and recent studies have tried to explore the mechanisms of gene-environment interactions (discussed further in Chapter 15).

It may also be pertinent to point out, this analysis doesn't consider iatrogenic factors as environmental influences to determine their role in explaining the heterogeneity in the clinical expression of PD but these are analysed in more detail in Chapter 14.

13.6 Conclusion

The subtypes of PD analysed in this thesis are not significantly influenced by environmental risk factors preceding the diagnosis of PD with the exception of solvent exposure being more commonly reported by males compared to females. This does not, however, take into consideration the influence of environmental factor such as drug effects, analysed in detail in the next chapter, on the clinical heterogeneity of PD or the fact that the predominant basis for the sub-typing could be due to internal biological factors rather than external influences.

Chapter 14. Iatrogenic influences causing variation in Parkinson's disease

14.1 Objectives

The primary objective of this chapter is to analyse the variation in the medication prescriptions (iatrogenic influence) in a cohort of patients with early onset Parkinson's disease from the PRoBaND study. A secondary objective is to explore system specific side effects, using the cardiovascular system as an example, of dopaminergic drugs and other medications prescribed for comorbid conditions. We analysed a separate incidental cohort of 360 Parkinson's disease patients specifically for cardiac side effects.

14.2 Introduction

Levodopa initially provides good symptomatic control of parkinsonian symptoms. As symptomatic control deteriorates other classes of anti-parkinsonian drugs including dopamine agonists, catechol-O-methyl transferase inhibitors, or monoamine oxidase type B inhibitors are then added as adjuvant therapy [479]. The variation in medication requirements is largely determined by disease severity and duration [480], however, we wished to explore this further to find whether the motor subtype, gender, age at onset of Parkinson's disease (PD) and heritability of the parkinsonian trait also contributed to the variation. Iatrogenic problems in PD can have several manifestations ranging from the well-recognised side effects of dopaminergic medications such as nausea and orthostatic symptoms in the short term to dyskinesia in the long term. This spectrum of iatrogenic adverse events seems to be expanding with more recently reported associations such as peripheral neuropathy , reversible corneal oedema and the dopamine dysregulation syndrome[481-483]. In order to explore the variation in system specific adverse effects we analysed a separate incidental cohort of patients, without any preselection bias of study protocol, for cardiac side effects of anti-parkinsonism drugs and other medications prescribed for comorbid conditions.

14.3 Methods

The assessment for iatrogenic influence in PD was done in two stages. Patients from the EOPD cohort of the PRoBaND study were the subject of analysis for the iatrogenic influences in PD in the first stage. They attended 6 monthly clinic visits as per study protocol described in detail in Chapter 3. They were asked to fill in questionnaires for demographic data and a medication list at baseline (visit 0). Levodopa equivalent daily doses (LEDD) in mg/day were calculated using the formula described in detail in Chapter 5.

In order to analyse the variation in the medication requirements as an indicator of iatrogenic influences, all cases with EOPD were classified in 4 ways: according to motor subtypes (TDPD, PIGD and 'Mixed'), gender, age at onset of PD symptoms and heritability of the Parkinsonian trait as described in Chapter 4.

Further analysis of the iatrogenic influences in PD was done using a different cohort of 360 recently attending patients at 3 movement disorder clinics across Glasgow to specifically look at the cardiac side effects induced by drug prescription, as an example of unintended detrimental side effects of therapy. The results of the latter study are presented as a stand-alone paper that was published in the journal *Parkinsonism and Related Disorders* and is added as an appendix to this chapter.

Statistical analysis:

Descriptive data are presented as median and inter-quartile range (IQR) as data in most instances failed tests of normality (Shapiro Wilk). Kruskal Wallis test was used for inter-group comparisons of numerical data in scenarios where cases where classified into 3 groups e.g. motor subtypes with post hoc comparisons between groups using Mann Whitney U if the Kruskal Wallis test statistic showed a significant difference between the 3 groups. Mann Whitney U test was used for inter-group comparisons of numerical data where cases were classified into 2 groups e.g. males and females. Chi square tests or Fisher's exact tests were used for comparing groups for categorical data as appropriate.

Generalised linear models, based on ANCOVA, were used with age ,disease duration and Hoehn Yahr stages as covariates to determine the effect of the motor subtype, gender, age at onset and hereditary versus sporadic parkinsonism (independent variables) on the LEDD (dependent variable). Hoehn Yahr staging was used as a covariate in preference to MDS-UPDRS part 3 scores as the latter are influenced by the time during the day these are recorded, before or after taking PD medications, 'on' or 'off' state and there is greater inter-individual variation in clinical scoring. Logarithmic transformations (base 10) of numerical data were used where tests of normality (Shapiro Wilk) failed.

All statistical analyses were two-sided and significance level was set at 5%. Statistical tests were performed using Prism software (*version 4.0 for Windows*; CA, USA) and SPSS software (version *23 for Windows*; IL, USA).

14.4 Results

276 patients had completed all questionnaires relevant to medication exposures analysis (Figure 14.1) and this is indicated in the relevant tables (Tables 14.1- 14.4). Their demographic details have been presented in Table 5.1 in Chapter 5.

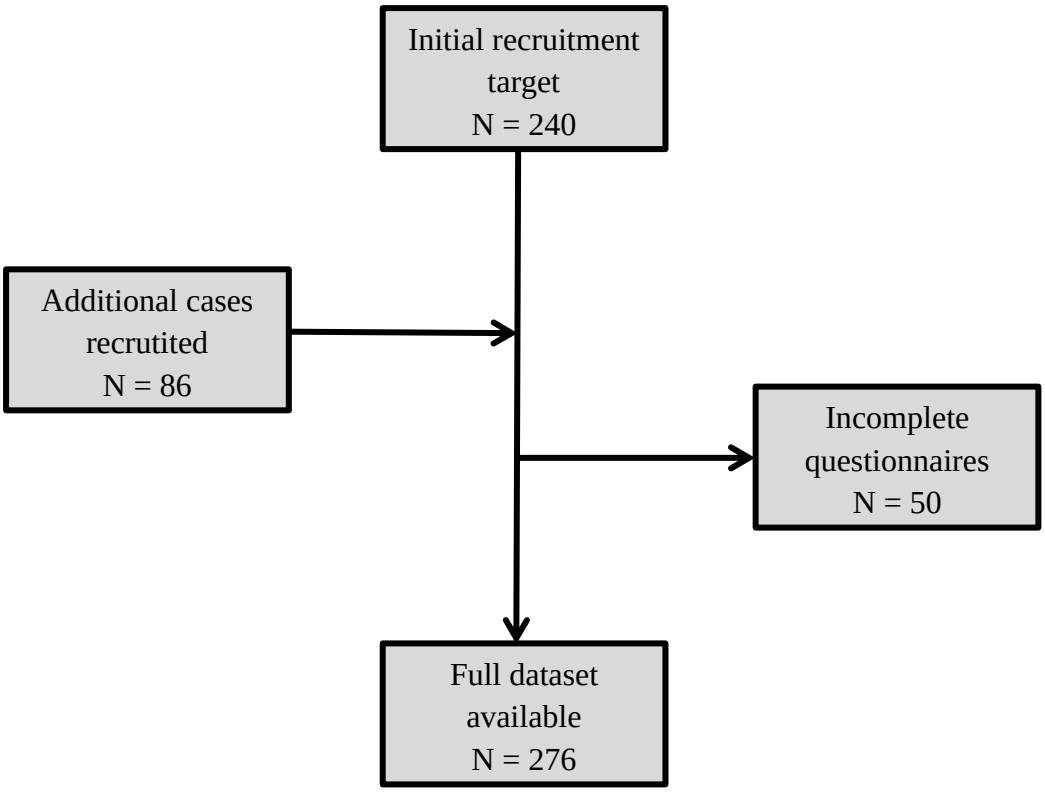

Figure 14.1 shows the disoposition of cases prior to data analysis for this chapter. No data imputation was allowed. Those who had incomplete questionnaires relevant to this analysis were not included.

The variation in anti-parkinsonian medication (PDMED) prescription as a risk factor for iatrogenic adverse events in these patients, classified by motor subtype, is presented in Table 14.1. There were statistically significant differences in PDMED prescription between the three motor subtypes ($p=0.003$) (Figure 14.2). Post hoc analysis showed the differences to be between TDPD and PIGD ($p<0.001$) but not between PIGD and 'Mixed' ($p= 0.616$) or between TDPD and 'Mixed' ($p=0.134$).

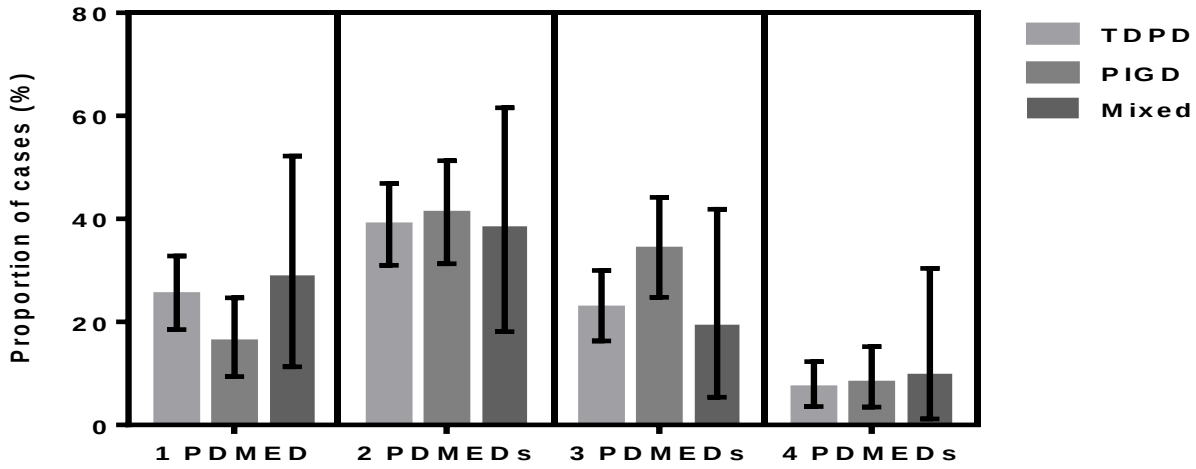

Figure 14.2 shows the proportion of patients who were prescribed one, two, three or four classes of PD medications (PDMED) (on the y-axis) and their motor subtype (on the x-axis).

Table 14.1 Variation in anti-parkinsonism medication exposure in cases (n=276) from the EOPD cohort classified by motor subtype.

Medications	**TDPD** median (IQR)	**PIGD** median (IQR)	**Mixed** median (IQR)	**p-value**
Number of cases	155	100	21	-
Age *(years)	50.4 (45.1-54.3)	54.6 (48.8-59.8)	49.6 (46.9-52.5)	<**0.001**
Sex (males)	64.1 %	59.0 %	65.0 %	0.356
Duration (years)	5.1 (2.0-9.6)	9.1 (4.6-15.0)	7.4 (2.5-11.8)	<**0.001**
L-DOPA	65.2 %	88.0 %	90.5 %	0.222
DA	45.8 %	49.0 %	33.3 %	0.669
MAOBI	45.8 %	39.0 %	33.3 %	0.385
COMTI	23.9 %	42.0 %	33.3 %	0.624
Amantadine	14.2 %	26.0 %	23.8 %	0.622
1 class of PDMED	25.2 %	16.0 %	28.6 %	0.172
2 classes of PDMED	38.7 %	41.0 %	38.1 %	0.927
3 classes of PDMED	22.6 %	34.0 %	19.0 %	0.095
4 classes of PDMED	7.1 %	8.0 %	9.5 %	0.909
LEDD (mg/day)	599 (300-965)	840 (532-1043)	738 (538-1053)	**0.003**
HY stage	2.0 (1.0-2.0)	2.0 (2.0-3.0)	1.5 (1.0-2.0)	<**0.001**

*** Age at registration, IQR = inter-quartile range. Data are presented in median (inter-quartile range) except where indicated, DA = dopamine agonists, MAOBI = monoamine oxidase B inhibitors, COMTI= catechol-o-methyl transferase inhibitors, PDMED = Parkinson's disease medication, LEDD = levodopa equivalent daily dose, HY = Hoehn Yahr stage.**

Classifying patients by gender showed no differences in the classes of anti-parkinsonsian medications prescribed (Figure 14.3) but more males were prescribed COMTI (p=0.016) while more females were prescribed Amantadine (p=0.011) (Table 14.2).

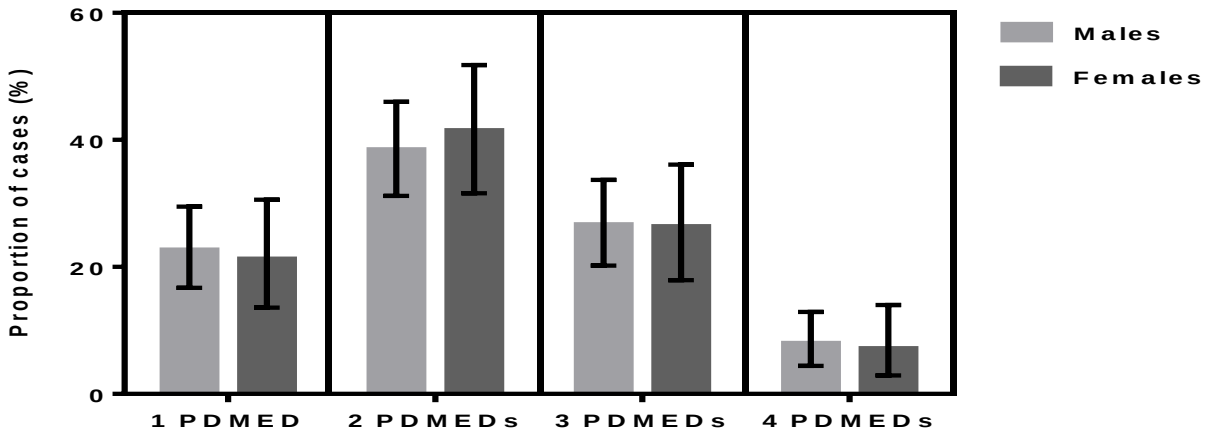

Figure 14.3 shows the proportion of patients who were prescribed one, two, three or four classes of PD medications (PDMED) classified by gender.

Table 14.2 Variation in anti-parkinsonism medication exposure in cases (n=276) from the EOPD cohort classified by gender.

Variable	**Males** median (IQR)	**Females** median (IQR)	**p-value**
Number of cases	177	99	-
Age*****(years)	51.7 (46.5-56.8)	51.1 (47.2-55.6)	0.968
Duration (years)	7.3 (2.8-11.5)	6.5(2.6-11.9)	0.737
L-DOPA	75.1 %	75.8 %	0.909
DA	42.9 %	51.5 %	0.170
MAOBI	41.2 %	44.4 %	0.606
COMTI	36.2 %	22.2 %	**0.016**
Amantadine	14.7 %	27.3 %	**0.011**
1 class of PDMED	22.6 %	21.2 %	0.790
2 classes of PDMED	38.4 %	41.4 %	0.625
3 classes of PDMED	26.6 %	26.3 %	0.958
4 classes of PDMED	7.9 %	7.1 %	0.801
LEDD (mg/day)	760 (400-1030)	600 (328-986)	0.453
HY stage	2.0 (1.5-2.5)	2.0 (1.3-2.5)	0.513

*** Age at registration, IQR = inter-quartile range. Data are presented in median (inter-quartile range) except where indicated, DA = dopamine agonists, MAOBI= monoamine oxidase B inhibitors, COMTI = catechol-o-methyl transferase inhibitors, PDMED = Parkinson's disease medication, LEDD = levodopa equivalent daily dose, HY = Hoehn Yahr stage.**

There were no differences in anti-parkinsonism medication prescription between those diagnosed with PD aged less than (or equal to) 40 years compared to those diagnosed after the age of 40 years (Figure 14.4) except more patients in the latter sub-group were prescribed COMTI (p=0.014) (Table 14.3).

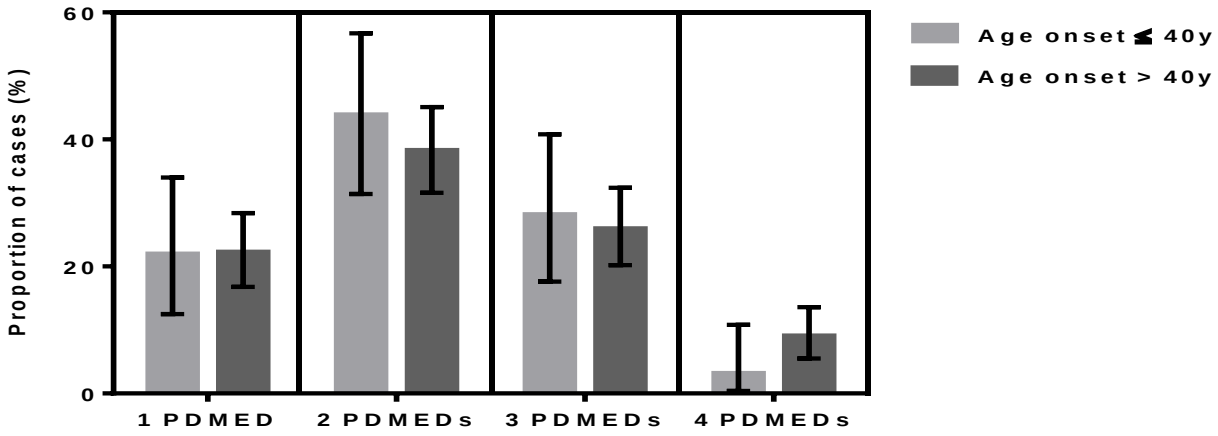

Figure 14.4 shows the proportion of patients who were prescribed one, two, three or four classes of PD medications (PDMED) classified by age at onset of PD.

Table 14.3 Variation in anti-parkinsonism medication exposure in cases (n=276) from the EOPD cohort classified by age at onset of PD.

Variable	**Age onset ≤ 40 yrs.** median (IQR)	**Age onset > 40 yrs.** median (IQR)	**p-value**
Number of cases	64	212	-
Age***** (years)	44.6 (40.2-50.8)	52.9 (48.8-57.0)	**<0.001**
Sex (males)	67.2 %	63.2 %	0.561
Duration (years)	7.9 (3.7-15.0)	6.6 (2.5-11.3)	0.707
L-DOPA	79.7 %	74.1 %	0.411
DA	46.9 %	45.8 %	0.887
MAOBI	46.9 %	41.0 %	0.408
COMTI	18.8 %	34.9 %	**0.014**
Amantadine	23.4 %	17.9 %	0.366
1 class of PDMED	21.9 %	22.2 %	0.870
2 classes of PDMED	43.8 %	38.2 %	0.427
3 classes of PDMED	28.1 %	25.9 %	0.748
4 classes of PDMED	3.1 %	9.0 %	0.178
LEDD (mg/day)	700 (363-998)	715 (400-1008)	0.171
HY stage	2.0 (1.0-2.5)	2.0 (1.5-2.5)	**0.016**

*** Age at registration, IQR = inter-quartile range. Data are presented in median (inter-quartile range) except where indicated, DA = dopamine agonists, MAOBI = monoamine oxidase B inhibitors, COMTI = catechol-o-methyl transferase inhibitors, PDMED = Parkinson's disease medication, LEDD = levodopa equivalent daily dose, HY = Hoehn Yahr stage**

There were no differences in anti-parkinsonism medication prescription between those with a positive family history of PD compared to those with a negative family history of PD (Figure 14.5) except more patients in the former sub-group were prescribed 3 classes of anti-parkinsonian medications (p=0.009) compared to the latter (Table 14.4).

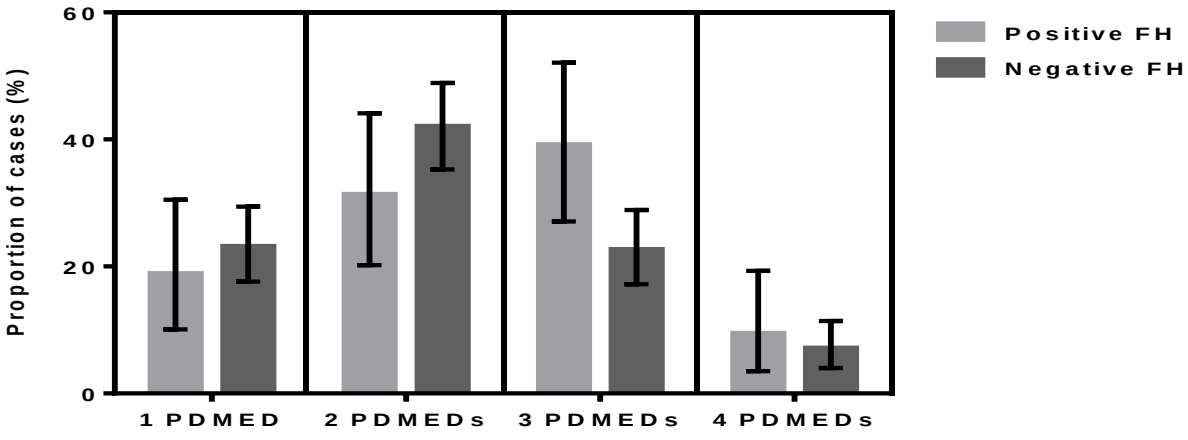

Figure 14.5 shows the proportion of patients who were prescribed one, two, three or four classes of PD medications (PDMED) classified by family history (FH) of PD.

Table 14.4 Variation in anti-parkinsonism medication exposure in cases (n=276) from the EOPD cohort classified by family history of PD.

Variable	**Positive FH** median (IQR)	**Negative FH** median (IQR)	**p-value**
Number of cases	64	212	-
Age***** (years)	52.2 (47.3-56.8)	51.2 (46.9-56.1)	0.582
Sex (males)	65.6 %	63.7 %	0.776
Duration (years)	6.7 (3.5-12.1)	6.8 (2.5-11.3)	0.312
L-DOPA	81.3 %	73.6 %	0.249
DA	43.8 %	46.7 %	0.679
MAOBI	59.4 %	37.3 %	0.002
COMTI	34.4 %	30.2 %	0.526
Amantadine	18.8 %	19.3 %	0.916
1 class of PDMED	18.8 %	23.1 %	0.607
2 classes of PDMED	31.3 %	42.0 %	0.124
3 classes of PDMED	39.1 %	22.6 %	**0.009**
4 classes of PDMED	9.4 %	7.1 %	0.591
LEDD (mg/day)	824 (460-1033)	655 (400-1000)	0.191
HY stage	2.0 (1.5-2.0)	2.0 (1.5-2.5)	0.583

FH = family history, * Age at registration, IQR = inter-quartile range. Data are presented in median (inter-quartile range) except where indicated, DA = dopamine agonists, MAOBI = monoamine oxidase B inhibitors, COMTI = catechol-o-methyl transferase inhibitors, PDMED = Parkinson's disease medication, LEDD = Levodopa equivalent daily dose, HY = Hoehn Yahr stage.

14.5 Discussion

Iatrogenic influences in the general population due the prescription of drugs with dopamine receptor blocking potential can cause drug induced parkinsonism (DIP) which is the most prevalent cause of secondary parkinsonism in clinical practice in the western world [484]. More importantly subclinical Lewy body disease in some cases may provide a pathologic

substrate for an individual's susceptibility to DIP and the persistence of parkinsonian symptoms even after the withdrawal of dopamine receptor blockers in such cases may represent drug unmasked parkinsonism (DUP). Latent disruption of the dopaminergic nigrostriatal projections, as evident on L-6-[F^{l8}] fluorodopa positron emission tomography (^{18}F-dopa PET), in DUP is different from DIP which has normal presynaptic dopaminergic uptake [485].

Drugs that are used to treat PD can have both dose related side effects as well as idiosyncratic and novel side effects such as dopamine dysregulation syndrome. Therefore, as an unintended consequence of the art of healing, physicians expose their patients to the double edged sword of anti-parkinsonism drugs. It is also reported that patients with PD are vulnerable to cardiac adverse effects of drugs due to inherent autonomic dysfunction compounded by similar side effects of a number of prescribed medications and this is analysed separately.

The prescription of these drugs is primarily determined by the motor disability of PD but influenced by other factors such as patient preference, side effects and drug interactions. In order to analyse the variation in prescription of these drugs to see whether this is influenced by the clinical subtypes of PD after adjusting for confounding variables such as age, disease duration and motor disability we classified patients as described in Chapter 3. Our results showed that the medication requirements in LEDD (mg/day), after adjusting for differences in age, disease duration and motor disability, were significantly greater in those with the PIGD phenotype and TDPD ($p<0.001$) but there were no significant differences in LEDD between the TDPD and 'Mixed' or PIGD ($p=0.616$) and 'Mixed'($p=0.134$) phenotypes. This is not surprising given that PIGD is less treatment responsive than the TDPD phenotype as levodopa had marked symptomatic effects on all features, but low potency for effect on PIGD (ED_{50} of 1237 mg/ day compared with 7–24 mg/ day for other motor and non-motor features) [486].

The influence of gender on medication requirements has not been directly investigated, however, several previous studies looking at gender differences in non-motor characteristics reported no differences in LEDD in males and females while analysing their baseline characteristics[327, 487]. Our results showed no significant differences in medication requirements in LEDD (mg/day), after adjusting for differences in age, disease duration and motor disability, between the two groups ($p=0.453$).

The influence of age at onset of PD on medication requirements (levodopa daily dose) was investigated in the Sydney multicentre study. There was no significant difference in the levodopa requirements at 5 years in those diagnosed with PD at age less than 70 years compared to those diagnosed with PD after the age of 70 years but this was not corrected for disease stage [488]. Another study comparing 43 patients with old onset (age onset >78 years) compared to 81 middle age onset (age onset 43-66 years) patients found no significant differences in the dose of levodopa or dopamine agonists between the 2 groups at 2 years but this was also not corrected for disease stage. We adjusted for disease duration and differences in UPDRS 3 scores and found no significant differences in medication requirements in LEDD (mg/day), after adjusting for differences in age, disease duration and motor disability, between the two groups (p=0.171).

The influence of family history of PD on medication requirements has not been investigated. Our results showed no significant differences in medication requirements in LEDD (mg/day), after adjusting for differences in age, disease duration and motor disability, between the two groups (p=0.191).

14.6 Conclusion

The major determinants of medication requirements in PD are age, disease duration and motor disability. Patients with the PIGD phenotype have motor symptoms that are less responsive to dopamine replacement and therefore require significantly greater anti-parkinsonian medications compared to TDPD. Levodopa was the most frequently prescribed medication (75% of cases) followed by dopamine agonists (60%), MAOBI (42%) and COMTI (32%). Most patients required 2 or 3 classes of PD medications at a median disease duration of 6.8 (2.7-11.5) years.

Original observations: Study of drug usage and iatrogenic influences on the cardiovascular system in PD.

Abstract

Introduction

Epidemiological studies report an association of ventricular arrhythmias with medication through prolongation of the cardiac QT interval. This has implications in the management of Parkinson's disease, as commonly prescribed drugs for non-motor symptoms and comorbidities have QT prolonging potential.

Objectives

To review prescribed medication in Parkinson's disease patients, in particular the use of drugs that may prolong the cardiac QT interval, in relation to other risk factors for QT prolongation.

Methods

Medication prescription and doses, presence of underlying cardiac disease, patient age, and sex were recorded in a cross-sectional sample of 360 current PD patients attending two district and one regional specialist hospital based movement disorder clinics.

Results

We sampled 360 consecutive patients with PD, median age 66.5 years (interquartile range 58.5-74.8) and median disease duration 4.2 years (interquartile range 1.2 -8.0 years). 125 (34.7%) were taking one or more drugs with definite potential to prolong QT, including domperidone in 91 (25.2%), citalopram or escitalopram in 47 (13.1%), and concurrent antibiotics in 5 (1.3%). Cofactors increasing the risk for QT interval prolongation were: age over 60 years 71.7%, female sex 46.9% and presence of cardiac disease 19.2%. In patients

with combined risk factors, the rate of prescription of at least one definite QT prolonging drug was between 34.5 and 42.1%.

Conclusion

Combination therapy and comorbidity relevant to cardiac QT prolongation are common in patients with Parkinson's disease. Strategies to reduce the proportion of patients at risk from iatrogenic adverse cardiac events are warranted.

Introduction

The therapeutic management of Parkinson's disease (PD) has broadened with increased recognition and treatment of non-motor symptoms affecting quality of life, such as depression and bladder dysfunction [489]. Epidemiological studies show an association of some of these treatments with cardiac arrhythmia, through prolongation of the corrected cardiac QT interval (QTc), resulting in torsades de pointes or ventricular tachycardia, which are often fatal [490, 491]. Those studies have led to regulatory alerts from government and federal drug regulatory agencies, in particular for citalopram and domperidone[492, 493]. In general terms, QT prolongation predicts cardiovascular mortality and may be an independent risk factor for stroke [494, 495]. The issue of drugs with adverse potential on cardiac conduction requires particular consideration, because of an inherent propensity to autonomic dysregulation in PD worsened by extraneous factors, which may collectively contribute to patient morbidity and even premature death. Concern about these drugs is not restricted to the rare congenital long QT syndromes caused by mutations in genes encoding cardiac ion channels. In the management of patients with Parkinson's disease, acquired factors of relevance which can impact on cardiac conduction include iatrogenic autonomic disturbances, cardiovascular comorbidities such as cardiac ischemia or ventricular hypertrophy, and the concurrent use of other drugs such as diuretics resulting in electrolyte imbalance [496].

Several classes of drugs are implicated in QTc prolongation, including anti-emetics, antidepressants, and antibiotics. Domperidone is frequently used in PD in Europe and elsewhere, both for its licensed antiemetic indication, and 'off-label' to treat symptomatic postural hypotension. It is not approved in the US, and was the subject of a 2004 warning by the Food and Drug Administration (FDA) against unregulated use to increase lactation, because of safety concerns regarding the cardiac QT interval. The association of domperidone in epidemiological studies with a small but significant increased risk (odds ratio 1.6-3.7) of ventricular arrhythmias and sudden cardiac death, resulted in safety updates in 2011-12 by the United Kingdom Medicines Health Regulatory Agency (MHRA), and Canada Health. The need to review the patient's on-going requirement for domperidone and to avoid doses higher than 30mg per day whenever possible, are emphasized.

Citalopram and escitalopram may also prolong the QT interval and a recent MHRA safety update has reduced the maximal safe dose of citalopram to 20mg daily in patients older than 65 years.

Combination of these agents with some antipsychotics (e.g. haloperidol), tricyclic antidepressants, antimicrobial agents (e.g. intravenous erythromycin), antihistamines (e.g. astemizole) and anti-retrovirals (e.g. ritonavir) have additive influences on QT interval prolongation.

A second FDA safety alert also highlights that patients with hepatic impairment, the elderly, poor CYP2C19 metabolizers (i.e. the cytochrome P450 isoform that metabolizes citalopram), or patients taking cimetidine or other CYP2C19 inhibitors should not receive citalopram above 20mg daily as the resulting increased citalopram concentration increase the risk of QTc prolongation and subsequent torsade de pointes.

We examined the usage of drugs with the potential to prolong the cardiac QT interval, and the presence of other risk factors, in a broadly representative sample of Parkinson's disease patients from the local community, to determine an estimate of the prevalence of this issue and if changes in clinical practice could improve patient safety.

Methods

Medication prescription and doses, presence of underlying cardiac disease, patient age, and sex were recorded in a cross-sectional sample of 360 current PD patients attending two district and one regional specialist hospital based movement disorder clinics.

Drugs with potential to prolong the cardiac QT interval were tabulated according to the classification as definite, possible, and conditional, according to the Arizona Center for Education and Research on Therapeutics (ACERT) which includes agents not approved by the FDA, and is recognised by other groups. Drugs that inhibit the metabolism of domperidone and citalopram, and increasing their arrhythmogenic potential from elevated plasma levels were also noted. Drugs relevant only to congenital QT syndromes e.g. salbutamol were not included.

Risk factors for cardiac QT interval prolongation besides drugs were used from the third national health and nutrition examination survey of the Food and Drug Administration (FDA) of the United Sates.

Fisher's exact test was used to compare groups, using Prism (*version 5.0*, GraphPad Software, CA, USA).

Results

We sampled 360 consecutive PD patients (191 male, 53 %), median age 66.5 years (interquartile range 58.5-74.8), median disease duration 4.2 years (interquartile range 1.2 -8.0 years) in this study. 125 (34.7%) were taking one or more drugs with definite potential to prolong QT, including domperidone in 91 (25.2%), citalopram or escitalopram in 47 (13.1%), and concurrent antibiotics in 5 (1.3%). Prescription rates for drugs with possible and conditional effects on QT were lower (Table 14.5).

Table 14.5 Number of drugs prescribed in 360 patients with Parkinson's disease, by drug class, according to risk grading for prolongation of the cardiac QT interval.

Drug class	**Potential for QT prolongation**			
	Definite	Possible	Conditional	Total N (%)
Antibiotics				
Clarithromycin	3			3
Erythromycin	2			2
Trimethoprim			9	9
Ciprofloxacin			3	3
sub-total	**5**		**12**	**17 (5.9)**
Cardiac				
Sotalol	2			2
Dronaderone		1		1
sub-total	**2**	**1**		**3 (1.4)**
Gastrointestinal				
Domperidone	91			**91 (31.4)**
Neurological				
Amantadine		30		**30 (10.3)**
Psychiatric				
Antidepressants				
Citalopram	46			46
Escitalopram	1			1
Venlafaxine		4		4
Amitriptyline			25	25
Fluoxetine			22	22
Sertraline			8	8
Dothiepin			4	4
Paroxetine			2	2
Trazodone			1	1
sub-total	**47**	**4**	**62**	**113 (39)**
Antipsychotics				
Quetiapine		11		11
Lithium		5		5
sub-total		**16**		**16 (5.5)**
Genitourinary				
Alfuzosin		3		3
Solifenacin			17	17
sub-total		**3**	**17**	**20 (6.9)**
Total number of drugs	145	54	91	290

One definite QT prolonging drug was prescribed in 106 patients (29.4%), while there was co-prescription of 2 such drugs in 19 patients (5.0%) and 3 such drugs in 1 patient (0.3%) (Table 14.6).

Table 14.6 Number of QT prolonging drugs prescribed per patient according to ACERT risk grading.

QT effect	**Number of QT prolonging drugs prescribed**			**Patients, n (%)**
	1	2	3	
Definite	106	18	1	125 (34.7%)
Possible	46	4	-	50 (13.9%)
Conditional	75	8	-	83 (23.1%)

ACERT= Arizona Center for Education and Research on Therapeutics

The prevalence of cofactors, individually and combined, and the number of drugs with definite QT prolonging effect are shown in Table 14.7. Cofactors increasing the risk for QT interval prolongation were: age greater than 60 years 71.7%, female sex 46.9%, and presence of cardiac disease 19.2%. Considering a combination of these risk factors, the rate of prescription of at least one definite QT prolonging drug was between 34.5 and 42.1%, although this involved progressively fewer patients (Table 14.7). While older patients were more likely to have cardiac disease (p=0.0234) and to be prescribed diuretics (p=0.0006), they were no more likely to be on drugs with definite or possible QT prolonging effects than younger patients.

Table 14.7 Individual and combined risk factors in patients (n=360) prescribed one or more drug with definite potential to prolong the cardiac QT interval

Risk factor	N (%)	Number of definite QT prolonging drugs			
		1	2	3	Total n (%)
Age over 60 years	262 (71.7%)	74	13	1	88 (33.6%)
Female	169 (46.9%)	54	13	0	67 (39.6%)
Cardiac disease	69 (19.2%)	19	2	0	21 (30.4%)
Over 60 + female	126 (35.0%)	40	10	0	50 (39.7%)
Over 60 + cardiac disease	58 (16.1%)	18	2	0	20 (34.5%)
Over 60 + female + cardiac disease	19 (5.3%)	7	1	0	8 (42.1%)

Four of 47 patients (8.5%) aged over 65 years were prescribed citalopram above the new recommended maximum of 20mg daily, and 16 of 91 (17.5%) were on domperidone exceeding 30mg daily. There was no evidence of increased prescription of domperidone in patients taking dopamine agonists (35/136, 25.7%) versus other anti-parkinsonian therapy (56/205, 27.3%), p=0.803.

Prescription of other medications with drug-drug interactions that may increase plasma levels of domperidone or citalopram through interaction with the hepatic cytochrome P450 system were absent in relation to domperidone, as no patients had concomitant use of agents metabolized by the CYP3A4 system (e.g. ketoconazole); however, 16 of 47 patients prescribed citalopram (34%) were co-prescribed agents that share its CYP2C19 metabolic pathway, consisting of proton pump inhibitors (omeprazole, esomeprazole, lansoprazole, pantoprazole) in 15/16 (93.8%) and modafinil in 1/16 (6.3%).

Discussion

Prolongation of the QT interval, which is usually multifactorial, can lead to torsades de pointes, a specific and often self-limiting arrhythmia which may progress to fatal ventricular arrest [497, 498]. The epidemiological association of co-prescription of more than one drug

with adverse cardiac events to PD patients, through the mechanism of cardiac QT prolongation and resultant arrhythmia, have not been previously examined in this population. Domperidone was the most frequently prescribed agent with definite potential to prolong the QT interval in our study. Patients taking domperidone at a daily dose exceeding 30mg are highlighted as a risk group in the MHRA safety update based on large epidemiological studies. A population-based case control study found that 10 of 1304 cases of sudden cardiac death were in domperidone treated patients, giving an odds ratio (OR) of domperidone for sudden cardiac death of 3.72 (95% CI 1.72-8.08) [492]. A retrospective case control study of 83212 individuals exposed to domperidone or a proton pump inhibitor found 1608 serious cardiac events (1559 sudden deaths; 49 ventricular arrhythmias), giving an OR for current domperidone use of 1.59 (95% CI 1.28-1.98) [493]. Domperidone is also the subject of 449 reports through the UK 'yellow card' system of adverse drug event reporting, which included 14 fatalities, half of those related to cardiac arrhythmia.

It has been widely prescribed to PD patients in licensed areas including Europe, as a first choice antiemetic to avoid central postsynaptic dopamine receptor blockade. It has also been frequently used to treat symptomatic orthostatic hypotension in PD. The presence of orthostatic hypotension has often, paradoxically, been a potential indication for increasing the dose of domperidone for symptomatic management. If such treatment contributes to adverse cardiac events from self-limiting torsades de pointes which manifests as syncopal episodes and is misinterpreted by the clinician as persistent or worsening symptomatic orthostatic hypotension, dose escalation of domperidone could aggravate an already problematic situation. In such a scenario, the symptoms arising from orthostatic hypotension and cardiac dysrhythmia can also have a broader phenotype than typical pre-syncopal or syncopal events, with episodes of generalized weakness and mental 'clouding' being recognised as manifestations [499].

Given the increased risks of cardiac adverse events from domperidone (>30 mg /day) in the presence of autonomic dysfunction caution is clearly indicated however the question arises as to the level of risk in patients when such features are absent.

In practical terms, the absence of orthostatic hypotension or syncope as markers of autonomic dysfunction does not remove the risk from drugs with potential to prolong the QT interval, as the mechanisms for peripheral and cardiac autonomic dysfunction differ; while around half of

those with orthostatic hypotension also have cardiac sympathetic dysfunction [500]. Randomized clinical trial evidence for domperidone having beneficial effects on orthostatic hypotension is very limited. In a crossover study with fludrocortisone in 17 patients without a placebo group, any positive effects noted were relative to non-pharmacological measurements in the run-in period of the study, and not performed on an intent-to-treat basis[501]. However, clinical experience of reduced side-effects when co-prescribing domperidone with dopamine agonists, in particular Apo morphine, is supported by autonomic measurements [502]. These competing elements of clinical management need to be considered in individual patient treatment decisions. Total daily doses of domperidone should be limited to 30 mg/day in PD patients and co-prescription of other QT prolonging drugs avoided.

Citalopram was the second commonest drug with QT prolonging effects in our cohort. It is widely used to treat depression, in patients with and without PD, and has a convenient dosing schedule, but the problem of QT interval prolongation and torsades de pointes even at conventional doses has emerged [503]. Other selective serotonin reuptake inhibitors have lesser potential to prolong the cardiac QT interval, and are therefore graded possible rather than definite in the QT interval prolonging drugs risk classification. The balance of benefits and risks of citalopram and escitalopram should be considered carefully, particularly at higher doses, in patients with pre-existing risk factors for QT interval prolongation, such as significant bradycardia, recent acute myocardial infarction, or decompensated heart failure [492].

Several classes of antimicrobial drugs including macrolides (e.g. erythromycin, azithromycin, and clarithromycin), azoles (e.g. fluconazole, ketoconazole) and fluoroquinolones (e.g. ciprofloxacin) also have the potential to prolong the cardiac QT interval and thereby increase the risk of torsades. Given the intermittency of antibiotic use, our point estimate of 1.3% based on prevailing antibiotic therapy at the time of last clinic review is an underestimate of such exposure, and the overall rate of 5.2% patients receiving 2 or more QT prolonging drugs concurrently is therefore likely to be an underestimate.

A case control study from a medical record database of 500000 people compared 775 cases of sudden cardiac death to 6297 matched controls; current use of non-cardiac QTc-prolonging

drugs was associated with an almost three-fold increased risk of sudden cardiac death [504]. Our study bridges the gap between such large epidemiological studies (none of which is specific to PD) and small PD studies examining drug effects on the cardiac QT interval. The findings from both types of study are relevant as a perspective to the current work.
These large studies indicate that the detection of transient self-terminating ventricular arrhythmia causing syncopal events (potentially from QT prolonging drugs), would be difficult in the setting of outpatient movement disorder clinics as there would be too few events to recognise or adequately quantify. However cardiovascular events are one of the 5 most frequent causes for hospital admission in PD patients [505]. While torsades de pointes is estimated to occur in 1-5% of patients treated with cardiac antiarrhythmic drugs that prolong QT, a much lower though inexactly quantified risk for non-cardiac drugs, that we studied, is expected, with epidemiological estimates of the population attributable risk of non-cardiac QTc-prolonging drugs being around 2% [504].

Although electrocardiographic recordings were not obtained in our study, which might be considered a limitation, they are perhaps surprisingly not central to the issues under consideration. ECG measurement is not definitive, as there is diurnal variation (often exceeding drug effects), correction is required for heart rate, and there are gender differences (a longer QT interval in females), meaning that the QT interval has varying definitions for normal, borderline, and prolonged [506, 507]. Further, a normal baseline QT interval (e.g. to screen pre-treatment) does not preclude the emergence of QT prolongation, exemplified by some congenital QT syndrome cases developing QT prolongation only while on an offending drug [508]. The large epidemiological studies used diagnostic coding for cardiac arrhythmia and sudden death, rather than ECG measurements, on the presumption that QT prolongation through drug exposure was the causative mechanism. ECG screening or monitoring is therefore not central to regulatory guidance [493]. Although it may be part of the assessment in patients with cardiac disease or cardiovascular symptoms, review of therapeutic need for particular treatments is the primary focus, noting upper dose limits (regardless of ECG findings) in relation to risk factors such as patient age. We therefore followed this practical approach in the present assessment of risk in PD patients. A prospective ECG study to provide more quantifiable information of the cardiac risk is now underway, to determine QT interval changes in relation to drugs, doses, and adjustments in therapy.

There is limited data on cardiac causes of death in PD. Autonomic effects or drug interactions were not considered causative of excess mortality found in one study in patients treated with levodopa and selegiline [161]. Prolonged cardiac QT intervals (451 and 470 msec in the year preceding death) in 2 of 48 PD patients who died suddenly were reported, but causation was not conclusive [509]. The QT interval has also been studied more directly in relation to autonomic function in PD patients. The QTc was prolonged in 30 PD patients, and correlated with the Valsalva ratio, patient age, and disease staging [510]. Similar observations regarding QTc and Valsalva, but not other markers of autonomic dysfunction, were made in 34 PD patients, selected to exclude cases with electrolyte imbalance, cardiac disease, or using QT prolonging drugs. Standard anti-parkinsonian medication did not affect the QT interval. Therefore caution is indicated in the prescription of other drugs for co- morbidities and non-motor symptoms of PD that have QT prolonging properties. Both direct and indirect effects of these drugs may lead to adverse cardiac events.

Conclusion

As risk factors for acquired QT prolongation are common in PD, extra caution is often required when making treatment decisions for non-motor symptoms of PD such as orthostatic hypotension or depression. Adding or increasing the dose of drugs which may prolong the cardiac QT interval, in particular domperidone and citalopram, requires care in PD patients, taking due consideration of comorbidity, and concomitant drug therapy. This is of particular concern in PD patients compared to the general population due to the added dimension of autonomic dysfunction in PD and related disorders.

Note: Additional references for this paper are available online in the journal: *Parkinsonism Relat Disord.* 2013 Jun; 19(6):586-9.

Chapter 15. Conclusions and future directions of PRoBaND

Parkinson's disease (PD) is a complex trait, and several molecular pathways and pathogenic mechanisms can lead to the clinical picture of parkinsonism. At the individual level there are several determinants of PD risk, and within populations, the causes of PD can be diverse [511]. Although mutations in many different genes are recognized to cause PD, these account for roughly about 10% or less of PD cases in the general population. Population based epidemiological studies suggest environmental factors may also be involved. Indeed, it may turn out that the interplay of environmental factors and our genetic makeup not only influences the risk of developing PD but also influences the variation in the phenotypic expression of PD.

Early onset Parkinson's disease (EOPD) is a recognised subset of patients with PD which differs from late onset Parkinson's disease (LOPD) patients in several ways. EOPD cases tend to have a more gradual progression of parkinsonian signs and symptoms, earlier appearance of levodopa related dyskinesia and dose related motor fluctuations, as well as the more frequent presentation to clinicians with dystonia as an early sign [512].

The seminal study of Zetusky et al established the idea of clinical subtyping of patients with PD because of prognostic implications. In this study of patients with idiopathic PD (n=334), deterioration in mental status was correlated with the PIGD subtype while the TDPD subtype was associated with relative preservation of mental status, earlier age at onset, family history of parkinsonism, and generally a more favourable prognosis [324]. Clinical subtyping of PD patients can be done in more than one way, to analyse the heterogeneity in their phenotypic characteristics [320].

With this in mind we subtyped patients according to their motor presentation, gender, age at onset and family history of PD. We analysed the clinical data for variation in the motor, non-motor, cognitive and quality of life domains of PD. We found heterogeneity in the phenotypic expression of PD across all domains, in some characteristics, while finding no statistically

significant differences in other variables. These differences are tabulated and presented in the relevant chapters in the thesis.

While recognising that some of these differences may not have a direct impact on the way we manage PD cases, the stated aim of this research was not to change clinical practice but to analyse, quantify and document the heterogeneity in PD as well as to test hypothetical models of the factors responsible for this heterogeneity.

The heterogeneity that we found in our EOPD cohort, is very likely to be an expression of the underlying differences in the mechanisms, both genetic and environmental, that culminate in the expression of disease in individual patients. This is the substrate on which a mix of iatrogenic factors in the form of dopaminergic and non-dopaminergic medications that these patients are prescribed generate a plethora of phenotypes, each with subtle differences from case to case but allowing for patients at a group level, to be classified under clinical subtypes.

The drivers of variation can be summarised under the following 5 headings:

i. Environmental influences

Although no single environmental agent besides MPTP has been conclusively linked to PD, the influence of several environmental agents, both positively linked to the risk of developing PD such as pesticides [468], heavy metals [469], solvents [470], rural living [473] and negatively linked to the risk of developing PD such as smoking [471], coffee [472], has come up in large scale epidemiological studies. Our research showed some differences in the exposure to environmental agents such as solvents and smoking amongst the various subgroups of patients, however, this was not in a case control study format, as our study did not recruit 'normal controls'. We wanted to determine whether environonmental influences could account for some of the variation in the phenotypic characteristics as epigenetic factors i.e. interact with genes to change the odds of modifying the clinical characteristics of this disease [513]. While this may turn out to be true but environmental influences on their own can not explain all the variation.

ii. Genetic influences

Mutations in several genes (*SNCA, LRRK2, VPS35, Parkin, PINK1, LRRK2, GBA* and *DJ-1*) can cause rare monogenic forms of the disease. Our research showed a prevalence of 10.1% of genetic cases of PD in the EOPD cohort randomly tested for mutations in 3 genes *Parkin, GBA* and *LRRK2*. Besides the mutation in these genes that are inherited in a Mendelian fashion recent work has focussed on, common variants of small effect size, called single nucleotide polymorphisms (SNP), at several loci identified in genome wide association studies (GWAS) which may not only modulate the risk for developing PD, but also influence the variation in its clinical characteristics [435]. Such influences include the recently reported findings of an association of certain SNPs and the age at onset of PD symptoms. The most robust association reported was for rs10889162 polymorphism in a transcription factor binding site -582 bp from CYP2J2 arachidonic acid epoxygenase. Each variant allele was associated with 5.04 years older diagnosis age of patients (95% confidence interval 2.28-7.80, p=0.0003) in 258 newly diagnosed non-hispanic caucasian cases in Washington state, USA [514]. There are hundreds and thousands of such SNPs at numerous loci that are the focus of current research in PD and the protocol of the PRoBaND envisages genotyping all DNA samples using the Illumina Human Core Exome array, supplemented with custom content. This will allow the analysis of approximately 250,000 common and 250,000 rare variants, across the human genome. These SNPs have been selected because of their previous implication in a range of neurodegenerative, neurologic, and psychiatric disorders.

iii. Gene-environment and gene-gene interactions

The interaction of genes and environmental influences modifying the risk of developing PD has been studied. In one study pairwise interactions between environmental exposures and 18 variants (16 SNPs and two variable number tandem repeats, or "VNTRs") in *SNCA*, *MAPT* and *LRRK2* genes, were investigated using data from 1098 PD cases and 1098 matched controls. Five pairwise interactions had uncorrected p-values < 0.05. These included pairings of pesticides and *SNCA* rs3775423 or *MAPT* rs4792891; coffee drinking and *MAPT* H1/H2 haplotype or *MAPT* rs16940806; alcohol drinking and MAPT rs2435211 [515].
Several other studies have analysed gene environment interactions using different sets of genetic markers [516, 517]. This is an active field of research and in the years to come will

shed more light into how these interactions influence the variation in the clinical characteristics of this disorder. The analysis of gene-environment interactions was not done at this stage in PRoBaND as this would require the analysis of dozens of SNPs across the genes linked to PD; as alluded to above sequencing for these SNPs will be done in the next phase of the study.

Besides gene environment interactions, current research also delves into the influence of gene-gene interactions in PD, for example, polymorphic variants in *MAPT* show a significant association with age of onset in individuals with *LRRK2* mutations [518]. This finding has been replicated in different populations [519] and indicates an association of mutation-affected protein domain and mutation-extrinsic genetic factors, suggesting that gene-gene interactions could influence the phenotypic heterogeneity observed in PD. Future research projects based on PRoBaND could analyse polymorphic variants in *MAPT* and other genes to study these influences.

iv. Iatrogenic influences

A significant contributor to variation in the clinical characteristics of PD, including motor complications such as dyskinesia and non-motor problems such as cognitive dysfunction and syncope, is the influence of prescribed medication. This includes medications specific for PD such as dopaminergic drugs and non-PD medications for comorbidities. We analysed the prescription of non-PD medications in a cohort of 360 patients to analyse system specific effects on the cardiovascular system and the results are presented at the end of Chapter 13.

v. Multiple 'hit' hypothesis

The focus of research has always been on the dopaminergic system as being 'hit' by the disease process in PD, however, evidence is emerging that other neurotransmitter systems are also 'hit' and contribute to the symptomatology of PD, particularly the non-motor aspects of this disease. Results of post mortem and molecular imaging studies reveal parallel degenerations of cortical noradrenaline (NA) and serotonin (5-HT) innervations, which may contribute to affective and cognitive changes of PD [520]. One could hypothesise that part of the variation in the clinical phenotype of PD may also result from how much the other

neurotransmitter systems are affected in different individuals and at different stages of PD. Looking at the depletion of non-dopaminergic systems in PD was not part of my remit in this research thesis but the imaging sub-studies of PRoBaND can provide a research platform for future scientific studies of how these influences contribute to the variation in the phenotypic expression of PD.

A single model of population attributable risk factors for causing PD and explaining the variation in the phenotypic expression of PD would have to include environmental, lifestyle and genetic factors, individually and collectively. Several variables such as environmental exposures to chemicals, lifestlye factors such as smoking and genetic factors that include both monogenic disorders such as *LRRK2* and polymorphic variants in other genes such as *MAPT* have been analysed by other groups using stepwise logistic regression [196]. Individually these influences contribute to small population attributable risks for PD but collectively they can contribute to a substantial proportion of the population attributable risk for developing PD [196].

In conclusion, there is a compelling need to further understand how all these influences converge and interact to bear upon the clinical expression of PD in individuals, their response to treatment, their risk of developing complications and prognosis. That PRoBaND can be a platform for this endeavour is a stated aim and future direction for this study and the researchers involved in it.

The study protocol has a lab based component to look for proteomic and genetic biomarkers and the study set up allows collaboration with other similar studies such as OPDC. Pooling data on the genetic cases of PD can lead to more detailed phenotype-genotype correlations given the number of genetic cases in individual studies may not be large enough.

The most important contribution, one could therefore argue, is that PRoBaND can provide access to a wealth of information and biological material about a very big cohort of cases, both EOPD and LOPD, for longitudinal follow up over years and decades which can help answer questions about the natural history, progression and prognosis of PD.

Limitations of methodology of the EOPD arm of the PRoBaND study

One could argue that the lack of a control group in this arm would be a serious limitation to analyse the prevalence of some of the non-motor symptoms such as loss of sense of smell or depression that can be unrelated to EOPD and are common in the general population. However, one needs to bear in mind that the primary research goal as stated in the opening pages of this thesis is not to estimate the prevalence of non-motor symptoms in EOPD *vis a vis* the general population or to determine whether smoking is a protective influence on EOPD but to analyse the variation within an EOPD cohort. The analytical approach adopted for the EOPD arm was to analyse intraclass differences within several subtypes of EOPD replicating previous work done in LOPD. Nevertheless, we acknowledge that the lack of probability sampling is an important and obvious limitation to our research because it will prevent us from making any generalisations about the EOPD population. In the main arm of the PRoBaND study, a ten times larger datset will be avaialbe (n= 2000), with a control group (n=750) who will be age and sex-matched. This will then provide ample opportunities to answer specific hypothetical questions that could not be answered using data from the EOPD arm of the study. While every opportunity has been taken to minimise bias, there will be certain inherent selection biases that will exist in every prospective or retrospective study with pre-defined inclusion ansd exclusion criteria as well as a personal interface between recruiter, usually a clinician, and the recruit, usually a patient with the exception of controls.

The accuracy of diagnosis of PD in life, when recruiting patients to studies, based on clinical criteria alone has been challenged and can not be absolute but when the Queen Square Brain Bank criteria (asymmetrical onset, no atypical features, and no possible etiology for another parkinsonian syndrome), are used as we have done in this study, the proportion of true PD cases correctly identified is close to 95% compared to their autopsy pathologic diagnosis [521]. While most recent studies have used these as part of their inclusion criteria for classifying cases as PD, the same can not be said about historical studies. This limitation is recognised as a significant problem but is not specific to our study, our aim of linking with the Brain Bank study will provide avenues for further analysing clincopathologic data to provide a means of better defining clinical criteria for PD and this may include imaging modalities as a surrogate for autopsy brain tissue specimens.

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1. **Malek N**, Grosset DG. Enhancing dopamine treatments: worth the effort? Neurodegener Dis Manag.2012;2(4):365-373

2. Grosset KA, **Malek N**, Morgan F, Grosset DG. Phase IIa randomized double-blind, placebo-controlled study of inhaled apomorphine as acute challenge for rescuing 'off' periods in patients with established Parkinson's disease. Eur J Neurol. 2013 Jan 28.

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