CHAPTER 1

GENERAL INTRODUCTION

1.1. PARKINSON’S DISEASE

Parkinson’s disease (PD; paralysis agitans) is a neurodegenerative disease of the substantia nigra (an area in the basal ganglia of the brain, which controls voluntary movements and helps to regulate mood). Dr. James Parkinson discovered this disease and documented its syndromes in his famous monograph, Figure 1.1. An Essay on the Shaking Palsy published in 1817 [1].

![AN ESSAY ON THE SHAKING PALSY.](image)

**SHAKING PALSY.** *(Paralysis Agitans.)*

Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace; the sense and intellects being uninjured.

Figure 1.1. James Parkinson’s classical essay on shaking palsy
Parkinson’s disease is the second most common neurodegenerative disease after Alzheimer. According to the United Nations, at least four million people are affected by PD worldwide. It is estimated that the prevalence and incident rates of PD in Europe is approximately 108 to 257/100,000 and 11 to 19/100,000 per year, respectively. In the older age groups (i.e. > 60 years) the rates of prevalence and incidence are much higher: 1280 to 1500/100,000 and 346/100,000, respectively [2]. It is expected that its prevalence and the anticipated social and economical burden related to it, increase in the next few decades as a result of both increased longevity and multiplication of effective therapies despite the two to five times higher mortality than age-matched controls [3].

Parkinson’s disease occurs when certain neurons of the brain, mostly in the substantia nigra die or become impaired. The symptoms of Parkinson's disease result from the loss of these dopamine-secreting (dopaminergic) cells and subsequent loss of melanin, secreted by the same cells, in the pars compacta region of the substantia nigra (also known as black substance). This leads to inhibition of the direct pathway of movement and activation of the indirect pathway of movement. Since the direct pathway facilitates movement and the indirect pathway inhibits movement, the loss of these cells leads to a hypokinetic movement disorder. The lack of dopamine results in an excessive inhibition of the thalamus, leading to hypokinesia [3].

1.1.1. MAJOR SYMPTOMS OF THE PARKINSON’S DISEASE

PD affects mainly the motor system and its cardinal symptoms are tremor, rigidity, akinesia, postural abnormalities and gait impairment [3]. In addition to the motor symptoms, mental disorders like depression and gastrointestinal dysfunction may occur; all of which considerably impair the quality of life of the patients [4].
1.1.1.1. Tremor

Tremor in PD has a number of characteristics that make it easy to differentiate from other causes of tremor: it is slow with frequency of 4 to 6Hz and affects asymmetrically upper and lower limbs [5].

1.1.1.2. Rigidity

Rigidity or increased stiffness of the muscles, frequently associated with cogwheeling, is a plastic, lead-pipe form of hypertonia that affects many muscles of the limbs and the trunk that are responsible for the typical stooped posture in PD patients.

1.1.1.3. Akinesia

Akinesia (lack of movement) is perhaps the most disabling symptom of PD that includes many features such as delayed motor initiation and slow performance of voluntary movements (bradykinesia), insufficiency of motion (hypokinesia), difficulty in reaching a target with a single continues movement, rapid fatigue with repetitive movements, inability to execute simultaneous and sequential actions [6].

1.1.1.4. Gait

Gait can also be altered in PD. Akinesia is particularly obvious during gait where it is responsible for the short, shuffling steps, reduced arm swing, hesitations in start, turning-around and sometimes leading to freezing phenomena [6].

Various combinations of PD symptoms, their severity, location and variability over the time in a particular patient generate a significant functional disability that tends to increase as the disease progresses [7].
Apart from the complex and constantly evolving pattern of these motor changes, there is considerable interpersonal heterogeneity of PD, making comparisons between individual patients a difficult task. Therefore, diagnosis and monitoring PD the based on clinical symptoms is a major challenge.

1.1.2. TREATMENTS OF PD AND ON/OFF FLUCTUATIONS

Currently the principle treatments include medications that mimic dopamine, compounds used to create dopamine in the brain (such as levodopa) and drugs that inhibit the breakdown of dopamine. Among the drugs, levodopa is the most commonly used for PD.

However, a major disabling symptom of chronic levodopa (LD) therapy is dyskinesia [8]. Dyskinesia generally occurs at the maximal benefit from a single LD dose (peak-dose dyskinesia) that can involve any body part with choreic or dystonic movements. As dyskinesia is a side effect of the levodopa therapy, it is often referred to as levodopa-induced dyskinesia (LID) [9]. The actual emergence of dyskinesia during the day depends on timing and quantity of each individual dose of levodopa and also to a lesser extent, depends on stress, food and many other factors [9]. Other chronic levodopa therapy related motor manifestations that may develop are motor fluctuations such as wearing-off, early-morning dystonia, delayed ON or no-ON response and eventually ON-OFF phenomena [10].

Two important and commonly used terms regarding the parkinsonian state of the patients are ON and OFF states. During the ON state, the medication (in particular levodopa) is active and motor performance of the patient is improved. OFF state is the period that starts when the effects of the medications wear off and PD symptoms reemerge.
Many of PD patients start to fluctuate between the ON and OFF states. Moreover, during the ON state patients may suffer from dyskinesia. Figure 1.2 shows a schematic of these ON/OFF fluctuations during the day. The physicians constantly need to adjust the dose and the time between each intake of the medications to maximize the period of ON state and minimize the periods of OFF and dyskinesia. Over the time the response to a fixed dose of the levodopa therapy decreases and as a result, the dose or the time between each intake needs to be adjusted. Clearly, to optimally adjust the treatments, knowing the exact periods of ON and OFF state during the day is invaluable to the physicians.

Figure 1.2. A schematic view of the levodopa fluctuations in the patients with PD
1.1.3. DEEP BRAIN STIMULATION

Recently Deep Brain Stimulation (DBS) in the sub-thalamic nucleus (STN) has been introduced as a treatment in patients with pharmacoresistant fluctuations. In a surgical procedure, through a small opening in the skull an electrode is implanted into the basal ganglia (Figure 1.3). Electrical stimulation through the electrode interferes with neural activity in the target area, which can alleviate parkinsonian signs.

After the surgery is completed, an expert calibrates the unit in order to maximize its effectiveness. The calibration includes setting the frequency, voltage and duty cycle of the square wave oscillator inside the neurostimulator. The neurostimulator is a pacemaker like device that contains a battery and circuitry to generate the electrical signals that through the electrode stimulates the targeted area in the brain. Currently Medtronic Inc. is the leader in producing neurostimulators and other components of the STN-DBS.

![Figure 1.3. Components of the STN-DBS stimulation system](image-url)
STN-DBS results in a significant reduction of the needed medication and in some cases patients can completely stop taking them [11], which in turn eliminates the problem of wearing-off with the levodopa therapy and can stop fluctuations and the dyskinesia.

The programming of the neurostimulator can take up to a year to achieve an optimal setting. Sometimes DBS is performed unilaterally on the side of the brain opposite to the side of the body most affected by the disease, but in many cases it is performed bilaterally in a single operation.

1.1.4. DIAGNOSIS/ASSESSMENT OF PD

Currently, motor assessment in PD is mainly based on historical information, home diaries and neurological examination during visits to the clinic. These methods clearly suffer from many drawbacks: data from these sources can be highly subjective, they rely on the patient’s memory and perception of his own symptoms and they depend on the physician’s experience in the field. Moreover, most of the patients may not be aware of mild tremor or dyskinesia. They may not necessarily understand medical terminology. They may unconsciously exaggerate or attenuate symptoms’ severity. Finally, short-term memory can be affected by PD [12].

In an attempt to solve these problems and to find more objective assessments, several rating scales have been designed and used [13]. Among them, the Unified Parkinson’s Disease Rating Scale (UPDRS) is the most widely used [14]. This rating scale tries to quantify selected symptoms and signs of parkinsonism in a 5-points scoring system (with 0 for no sign and 4 for a marked severity of the sign).
Unfortunately, the UPDRS like any other semi-objective rating scale has limitations like intra and inter-observer inconsistencies, can be time consuming and can be biased by subjectivity issues related to historical information. Moreover, the pattern and severity of PD symptoms may vary considerably during the day, while clinical rating scales only provide moment-to-moment assessments and finally, measurements of motor functions made in the clinic may not accurately reflect the actual functional disability experienced by the patients in their daily life [15].

In addition to rating scales, akinesia and gait are sometimes evaluated by means of timed motor performance test [16], Purdue Pegboard test [17], pronation-supination test, hand movement between two points [18], finger dexterity [19], stand-walk-sit test [20] or tremor amplitude [21]. Also, objective methods have been suggested to quantify rigidity [22]. While these methods are quantitative, again they only provide information limited to the setting of the clinic.

Electromyography (EMG) techniques provide detection and monitoring of electrical muscle activities by attaching surface electrodes on the belly of the selected muscles. EMG does not directly measure movements and a large number of electrodes may be needed to study complex movements. EMG has been used for a long time to study tremor in PD and several ambulatory, long-term EMG recording systems have been described [23]. Also, ambulatory EMG recording has been used to detect basic body postures [24]. And finally, EMG has been used to study gait in PD [25], though not in ambulatory conditions.

An ambulatory approach to analyze gait based on special footwear with foot-switches or other pressure sensitive devices inside [26]. However, using special footwear is not always possible and may hinder subject’s normal gait. Moreover, PD patients may tend to shuffle while walking, making the initial and terminal
contact detection difficult. In these cases, the gait temporal parameters may not be calculated precisely. In addition, the foot-switch techniques do not provide spatial parameters.

Recent developments in microelectronics have led to design and production of a new generation of small, cheap and robust sensors that can be used to measure kinematic parameters of the movements of the body segments. These developments have breathed a new life in design of ambulatory systems for long-term monitoring of body movements. Accelerometers and gyroscopes have been used to detect and quantify tremor [27], bradykinesia and hypokinesia [6] in PD patients. Ambulatory gait analysis systems have been design based on accelerometers [28] and gyroscopes [29] for healthy subjects, elderly and pathological cases. These sensors have been used as activity monitor [30] or to classify different body postures [6]. Also, recently kinematic sensor has been used in detection and quantification of dyskinesia [31].

Today, especially regarding diagnosis of PD, none of the above-mentioned techniques are perfect. These methods are yet young and none of them has been used in large scale nor has reached consensus as a gold standard in the scientific and clinical community. Moreover, each system only either addresses one or few of the major PD motor dysfunctions. Finally, available ambulatory methods cannot detect individual PD patients with acceptable accuracy [32]. However, the diagnosis through imaging techniques such as cranial SPECT and PET are quite reliable but the accessibility of these imaging systems for developing and under developing country is considerable low. Hence, there is an increase need for the early and feasible diagnostic method for PD.
1.2. OBJECTIVES OF THE STUDY

The primary objective of this research is:

- To identify the molecular biomarkers for the diagnosis of PD from peripheral blood tissue by post genomic approaches.

Additional goal of this research is:

- To determine the potential lead molecules for PD to avoid adverse effects of levodopa treatment.

1.3. OUTLINE OF THE THESIS

The primary aim of this research is to identify the molecular biomarkers for the diagnosis of PD from peripheral blood tissue. The molecular based diagnosis will certainly improve the accuracy of diagnosis irrespective of physician’s or technician experience and also it is feasible for developing and under developing countries. This thesis is organized into five chapters. The first (current) chapter, Introduction, the basic ideas, provides a short review of the literature to discuss about the objectives of the research. Further, a detailed literature review concerning PD aetiology, diagnosis and treatment, with a particular emphasis on the possible role of current diagnosis. In Chapter 2, the metallomics based diagnosis of PD from the blood serum was analyzed. The aims, research questions, hypotheses and methods including their results and discussion are outlined. Chapter 3, deals about the FTIR spectral analysis of PD plasma and their outcome. Similarly, In Chapter 4, a metabolic profiling PD patient from human blood plasma was analyzed and reports a test reliability of identified biomarkers through the systems biological approach and their outcome was discussed. In Chapter 5, Insilco drug design, describes the QSAR and pharmacophore methods to determine the potential drug for Parkinson’s disease to minimize the adverse levodopa drug effect. Chapter 6, presents an summary and conclusions, which cover the thesis as a whole and put the
results into a public health context. Avenues for further research in this area are also discussed. All chapters of the thesis follow a similar structure. Each chapter starts with an introduction to bring the subject of the chapter into focus and it is followed by the details of the method, results and discussion. At the end of the thesis, in the bibliography section, all of the referenced articles, books and our resources used throughout the thesis are listed.

1.4. LITERATURE REVIEW

1.4.1. Definition and symptoms of PD

The disease was named “Parkinson’s Disease” after James Parkinson, who wrote the first published, detailed description of the disease in his “An Essay on the Shaking Palsy” [1]. The disease is often referred to as ‘idiopathic parkinsonism’ to differentiate the disease from other similar conditions and syndromes, such as drug-induced parkinsonism. The clinical picture of PD can be complex and quite variable between affected individuals in terms of age of onset, symptoms experienced, and rate of disease progression [33].

The cardinal features of PD include resting tremor, akinesia (defective movement initiation), bradykinesia (slow movement), muscular rigidity and postural instability. These symptoms are usually asymmetric. Diagnosis is made by clinical assessment based on the presence of the cardinal features. Pathological confirmation is only available on autopsy, and is based on the presence of Lewy bodies in particular regions of the brain and reduced numbers of dopaminergic neurons in the substantia nigra pars compacta region of the brain [34]. Standard diagnostic criteria, such as the Unified Parkinson’s Disease Rating Scale (UPDRS) is often used, although practitioners may modify these or employ their own criteria. Studies of diagnostic accuracy suggest many affected individuals are misdiagnosed.
A clinico-pathological study of neurologist-diagnosed PD cases found that according to autopsy results, 24% had been misdiagnosed [35]. A lower rate of diagnostic accuracy can be expected in community-based samples where many people with PD may not have been examined by a neurologist. A community-based study of patients recruited from general medical practices found that only 53% of those diagnosed with PD had clinically probable PD according to standard diagnostic criteria applied by a neurologist [36]. The highest accuracy of clinical diagnosis is achieved by specialist clinicians employing standard diagnostic criteria supplemented by long-term clinical observation [37].

In addition to the cardinal features, other ‘motor’ symptoms include micrographia (small handwriting), a mask-like facial expression and a small shuffling walk. Cognitive symptoms, such as memory disturbances, and particularly depression, are experienced by many people with PD [38]. Dementia is also reported to be higher amongst people with PD than in the general population of the same age [39].

Sensory disturbances, such as pain, tingling, numbness and burning have also been reported in many people with PD. Up to 90% of people with PD report a decreased sense of smell [40]. Other symptoms experienced by people with PD can include orthostatic hypotension (low blood pressure induced by standing upright), chronic gastrointestinal problems such as nausea, abdominal pain, and bloating[41], sexual dysfunction, dermatitis, excessive sweating, and urinary problems[40]. There is significant variability in the symptoms exhibited by people with PD and new symptoms may develop as the disease progresses [42].
1.4.2. PATHOPHYSIOLOGY

The most prominent pathological lesion observed in PD occurs in the basal ganglia of the brain’s extra-pyramidal system. The disease is characterized by a gradual loss of pigmented cells, mainly in the compact zone of the substantia nigra. These pigmented cells produce and store the neurotransmitter dopamine [43]. While a loss of dopaminergic cells is also seen in the normal ageing process, the loss of cells in people with PD is highly selective [44], following a characteristic pattern [43], and far more dramatic. Why this selective neuronal loss occurs in PD is still unclear, although oxidative stress mechanisms are possibly involved [45]. This loss of dopaminergic neurons results in a deficit of dopamine in all the components of the basal ganglia [43]. Reductions of dopamine by more than 95% in the putamen and 80% in the caudate nucleus are typically seen in people with PD and represent a defining biochemical feature of the disease [43].

It is interesting to note that before the disease becomes clinically evident as a motor disorder, the striatal dopamine loss must reach a critical value between 70-80% [43]. This lag in disease expression is explained by the existence of a compensatory mechanism whereby the remaining dopaminergic neurones increase their dopamine turnover (synthesis and release) and postsynaptic dopamine receptors become hypersensitive [43]. This finding is also consistent with the generally accepted theory that the underlying disease process is present for some time before diagnosis. During this ‘presymptomatic’ phase, people with PD may experience non-motor symptoms, such as depression or loss of olfaction, which have been reported to occur in people with PD up to 10-20 years before their ‘onset’ of PD [40]. A long pre-symptomatic period has important implications for the design of studies of PD aetiology. It may be difficult to distinguish which exposures occurred after the commencement of the disease process. Consideration must be
given to exposures from many years prior to commencement of symptoms, which may be more relevant than recent exposures.

Other neuronal systems are also affected and deteriorate to different degrees as the disease progresses [46]. As such, people with PD vary in their clinical presentation. The classic triad of motor symptoms (resting tremor, bradykinesia and rigidity), is mainly due to degeneration of ventral mesencephalic dopaminergic systems, whereas early cognitive deficits (frontal syndrome and depression) may arise from subcortical lesions. Between 15-30% of people with PD develop a dementia syndrome which may be due to damage to the cerebral cortex [43].

The rate of dopaminergic neuronal degeneration differs between people with PD, although cell loss continues throughout the course of the disease. Cell loss has been estimated to range from being extremely severe (90%) over short periods of time to relatively mild (60%) after many years of illness, in different affected individuals [46]. Studies of disease progression in PD by Hoehn and Yahr [7] and Marttila and Rinne [47] have reported a marked variability in the rate of progression between people with PD. Some were confined to a bed or wheelchair within 3 years of onset, while others only experienced minimal or no functional impairment after 10 years with the illness [40].

1.4.2.1. Oxidative Stress

Oxidative stress results when reduced oxygen species are formed in excessive amounts, producing cytotoxicity. Oxidative stress from either exogenous or endogenous neurotoxins has been suggested as one of the mechanism for the accelerated neuronal degeneration seen in PD. A number of studies support the role of oxidative stress in the degeneration of dopaminergic neurons, which have been
consistently shown to be particularly vulnerable to reactive oxygen species [48]. Mitochondrial dysfunction has been reported in a large percentage of people with PD [45], which gives support to the oxidative stress theory since reduced oxygen species are produced as intermediates in the production of cellular energy [49]. A number of markers of oxidative stress, such as decreased glutathione levels and increased levels of malondialdehyde and lipid hydroperoxidation, have also been reported in brains of people with PD [45, 49, 50].

The discovery that the chemical MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) can cause parkinsonism also supports an oxidative cause of PD[45]. MPTP binds with high affinity to the extra-neural enzyme, monoamine oxidase (MAO) type B, after traversing the blood brain barrier. This oxidation process transforms MPTP to the toxic pyridinium metabolite, MPP+ (1-methyl-4-phenylpyridinium). MPP+ then binds melanin in the dopaminergic neurons in sufficient concentration to destroy the cells [51]. The resulting syndrome is clinically indistinguishable from idiopathic PD and produces an akinetic-rigid state.

Environmental exposures may be involved in the aetiology of PD through oxidative stress mechanisms, as environmental pollutants including nitrogen dioxide (from vehicle exhaust fumes), halogenated hydrocarbons and pesticides may cause free radical reactions, either spontaneously or by active intermediates produced through their metabolism by the cytochrome P-450 enzyme system [52].

1.4.2.3. Apoptosis

Apoptosis is a mode of cell death by which cells are programmed to 'suicide' by forming fragments that are phagocytised by other cells. While apoptosis is a normal functional process by which certain cells are removed, it may also be involved in various disease pathologies. Dysfunction in the regulation of apoptotic cell death
can cause cells to die inappropriately, too much, or not at all [53]. Apoptosis has been suggested as the mode of death of cells in PD [49]. It has been suggested that apoptosis may be chemically induced by the accumulation of free radicals generated either outside or inside the cell [53], which is consistent with the oxidative stress theory.

1.4.2.4. Inflammation

A growing body of literature recognizes the role of inflammation of the brain (neuroinflammation) in the pathogenesis of PD. Post-mortem examination of the brains of people with PD have revealed the presence of pro-inflammatory factors, including complement proteins and cytokines [54]. Neuroinflammation can be induced by exposure to infectious agents, such as viruses, or to toxic substances. For example, the toxicity of rotenone to dopaminergic neurons is greatly increased by interactions with microglia, the resident immune cells in the brain involved with inflammation. Rotenone appears to activate microglia to produce free [55] and has been shown to interact synergistically with an inflammogen (lipopolysaccharide) to induce dopaminergic neurodegeneration. The involvement of activated microglia releasing free radicals and inflammatory cytokines in the neuroinflammation process is also consistent with the oxidative stress theory [56].

1.4.3. PREVALENCE AND INCIDENCE OF PD

PD is found worldwide and in all races [57]. The crude prevalence of PD ranges markedly between studies [58, 59]. The average adjusted prevalence ratio reported by a review of prevalence studies was 103 per 100,000 [57]. Differences in estimated prevalence may be due to true geographical variations, variance amongst studies in case definition and ascertainment, differences in population age structure, or a combination of these factors. Previous studies have suggested that Oriental races appear to have the lowest prevalence and Caucasians the highest [60].
However, these conclusions are based on studies from the 1980s in Chinese populations [61]. Since then other studies in China have reported prevalence rates equivalent to those reported in Western populations [62]. This could be due to a true increase in prevalence since these earlier studies, such as through improvement in life-expectancy of people with PD or an increase in incidence, or may be an artifact due to methodological differences between studies. Studies examining different racial groups within one geographical area have reported conflicting results. Schoenberg et al. [63] reported no difference in age and sex-adjusted prevalence between races, while Mayeux et al. [64] reported a lower age-adjusted prevalence of PD in African-American men compared to Caucasian or Hispanic men. Geographical variation in PD prevalence or mortality within homogeneous populations has also been reported [65-67], with some studies noting higher prevalence in rural compared to urban areas [68, 69].

Many different study designs have been used to assess PD prevalence and incidence. These include cross-sectional studies, longitudinal cross-sectional studies, drug consumption studies, medical record audits, and door-to-door surveys. In general, the door-to-door survey is accepted as the gold-standard design for estimation of the prevalence of PD, however even these surveys vary greatly in their methods, which limits their comparability. Door-to-door surveys can detect previously undiagnosed cases and have generally reported a greater prevalence of PD compared to studies using other methods, such as surveys of general practitioners [70]. However, door-to-door surveys are costly and time-consuming and so usually cover smaller areas than other methods [71]. If there are differences in the geographic distribution of PD cases, then the prevalence may be over or under-estimated if results are extrapolated to the entire population. Drug consumption studies can also be influenced by multiple confounders, such as treatment practices and the introduction of new drugs. These studies also miss those not treated with conventional pharmaceutical treatments for PD.
1.4.4. AGE-RELATED FACTORS

PD is generally considered a disease of the elderly and occurrence in younger age groups (less than 40 years) is rare. Most PD prevalence studies show a steadily increasing prevalence with age [72, 73]. Some also show a peak prevalence followed by a decrease in the oldest age groups such as 80 years and over; however, this may be due to lower numbers of participants in the oldest age groups resulting in unstable prevalence estimates, or a survivor effect [74-76]. Generally, door-to-door surveys, which are the most accurate in estimating prevalence, do not report a drop in prevalence in the oldest age range [77]. Similarly, most incidence studies also show steadily increasing incidence with increasing age [78]. Some studies also show a reduced incidence in the oldest age groups [61, 69]. This may be due to a real decrease in incidence or decreased case-finding in the very elderly who may be less likely to participate in surveys or are afflicted by other conditions that make diagnosis of PD more difficult, less of a priority, or result in mortality prior to PD diagnosis.

1.4.5. GENDER-RELATED FACTORS

A number of studies have reported that more males than females are affected by PD. However, given that initial reports of a male preponderance were derived from non-population based studies, which are subjected to biases that may distort sex distributions, the result has received criticism [79]. The differences among the study designs used makes it difficult to compare results, however most prevalence studies have reported a higher prevalence in males than females by up to 1.7:1.0 [69, 72]. Other studies report no difference in prevalence between males and females [59, 80] or higher prevalence in females [81]. Interestingly, some studies have reported the difference in prevalence of PD between males and females decreases at older age groups. This could be due to longer life-expectancy of
women with PD or less sex-related differences (due to post-menopausal decrease in female hormones) in incidence at older ages.

Gender-related differences in incidence may be more informative than prevalence for generating aetiological clues, given that prevalence may be reflective of increased mortality in one gender rather than higher incidence. Indeed, higher mortality in women with PD compared to men has been reported, which could explain the higher male prevalence of the disease [82].

1.4.5.1. Oestrogen

There is some evidence that oestrogen is responsible for gender-related differences in risk of PD and symptom profiles in people with PD. Oestrogen has been shown to modulate nigrostriatal dopaminergic activity and to exert a neuroprotective effect in animal models of PD [83, 84]. Some studies report more hysterectomies, surgically-induced menopause, and early menopause in women who subsequently develop PD compared to those who do not [85] while other studies report no difference in the time or type of menopause (natural or induced) between PD cases and controls [86, 87] and other studies have reported reduced risk with early menopause [88]. The differences in results may be due to a difference in the study populations, such as whether hormone replacement therapy was routinely used by women undergoing early menopause in one group or not.

1.4.6. GENETIC FACTORS

Although generally considered a sporadic disease (>80% of people with PD do not claim a family history [89], there is evidence to suggest that genetic factors play a role, at least in part, in the development of PD. Family history of PD is consistently associated with increased risk of PD in epidemiological studies [89-91]. A number of studies have described familial clustering of PD cases as evidence
of a genetic cause [92]. While common exposure to environmental factors has also been cited as another possible explanation [93], shared environment is not sufficient to explain the increased prevalence of PD in relatives with different residential histories. The findings of Uitti et al. [94] suggest that family history may play a greater role than previously thought. The study found the prevalence of familial cases of PD is likely to be significantly higher than previously reported due to inaccuracy of family histories obtained from people with PD and that the prevalence rate in relatives of affected individuals was up to five times higher than in the general population.

1.4.6.1. Genetic mutations

A number of specific genetic abnormalities and several chromosomal loci have been linked to rare forms of familial parkinsonism. Implicated genes that have been identified include Alpha-synuclein, Parkin, Ubiquitin carboxy-terminal hydrolase L1 (UCHL1), PTEN-induced putative kinase 1 (PINK1), DJ-1 and Leucine-rich repeat kinase 2 (LRRK2).

These genetic loci and their features of mutation are outlined in Table 1.1. In addition to these, a number of other loci believed to confer higher susceptibility to PD are being characterised via family studies [95, 95].
Table 1.1. Genetic association

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Gene Loci</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>Mutation in alpha synuclein gene on chromosome 4q21</td>
</tr>
<tr>
<td>PARK2</td>
<td>Mutation in the parkin gene on chromosome 6q25.2-q27</td>
</tr>
<tr>
<td>PARK3</td>
<td>Linkage to chromosome 2p13</td>
</tr>
<tr>
<td>PARK4</td>
<td>Triplication of alpha-synuclein gene</td>
</tr>
<tr>
<td>PARK5</td>
<td>Mutation in the UCHL1 gene on chromosome 4p14</td>
</tr>
<tr>
<td>PARK6</td>
<td>Mutation in the PINK1 gene on 1p35-36</td>
</tr>
<tr>
<td>PARK7</td>
<td>Mutation in the DJ1 gene on chromosome 1p36</td>
</tr>
<tr>
<td>PARK8</td>
<td>Mutations in the LRRK 2 gene on chromosome 12q12</td>
</tr>
<tr>
<td>PARK9</td>
<td>D1S2843 on chromosome 1p36</td>
</tr>
<tr>
<td>PARK10</td>
<td>Gene on chromosome 1p32</td>
</tr>
</tbody>
</table>

1.4.6.2. Genetic susceptibility

Various studies have also investigated whether common genetic variants can confer differential risk for Parkinson’s disease in individuals with sporadic disease. Candidate genes examined have included the PARK loci (Table 1.4.8.1) and genes involved with xenobiotic metabolism. In addition to familial parkinsonism caused by parkin gene mutations, polymorphisms in the parkin gene have also been associated with increased susceptibility to sporadic PD. Similarly, polymorphisms in the UCH-L1 gene alter susceptibility to sporadic PD, such as a polymorphic variant of UCH-L1 (S18Y), which is associated with decreased risk for PD [97, 98].
1.4.6.3. Family history of other neurodegenerative diseases

Family studies have confirmed that genetic risk factors also play an important role in the aetiology of neurodegenerative diseases other than PD, such as Alzheimer’s disease [99]. Some of these genetic risk factors might overlap with those for PD. For example, the apolipoprotein E4 genotype is a major genetic risk factor for AD [100] and has also been linked to earlier age of onset and increased risk of dementia in people with PD [101]. A number of studies have demonstrated an increased risk of PD associated with family history of Alzheimer’s disease and vice versa [102, 103].

An increased risk of Down’s syndrome (Trisomy 21) has been observed in relatives of people with Alzheimer’s disease and vice versa [99] Additionally, dementia of the Alzheimer’s disease type, with accompanying pathology, is common in people with Down’s syndrome, particularly after the age of 40 [104]. Extra-pyramidal signs, similar to the parkinsonian features observed in advanced Alzheimer’s disease, have also been observed in people with Down’s syndrome with dementia [105] and olfactory dysfunction is common to Down’s syndrome, Alzheimer’s disease and PD [106]. Lewy body pathology in the substantia nigra in addition to cortical Alzheimer-type pathology in people with Down’s syndrome has been reported [107]. However, few studies have examined family history of Down’s syndrome in people with PD.

1.4.7. MEDICAL HISTORY

1.4.7.1. Head injury

Case reports of acute severe cranial trauma describe a progressive parkinsonian syndrome in some patients [108, 109]. Also, repeated head trauma, such as experienced in the sport of boxing, has been shown to cause parkinsonism
[110, 111]. A number of epidemiological studies have also linked self-reported head injury to risk of PD [112, 113]. Conversely, many studies have not been able to establish this relationship [114, 115].

The main criticism of retrospective studies of head injury and PD has been the possibility of recall bias. Studies using medical records avoid this potential bias, but may introduce selection bias. The medical records linkage system in Olmsted County, Minnesota, which was developed as part of the Rochester Epidemiological project, largely avoids selection bias as it has included the lifelong medical records of virtually every resident in the county since the 1960s. This medical records linkage system was used in two epidemiological studies consisting of a longitudinal follow-up study and a case-control study, of head injury and PD. The follow-up study of head trauma patients did not find an increased standardised morbidity rate for PD in this group compared to the general population [116]. However, the case-control study which assessed episodes of head trauma preceding PD onset, reported an elevated odds of PD with history of mild head trauma with loss of consciousness or a more severe trauma (OR of 11.0, 95% CI = 1.4 to 85.2). Head trauma resulting in hospitalization was also more frequent in cases than in control subjects (OR = 8.0; 95% CI = 1.0 to 64.0) [117]. The different results in these studies may be due to study design. As head trauma is a relatively rare event [117], there may have been insufficient number of participants with serious head injury in the follow-up study to obtain a stable estimate of PD risk, as acknowledged by the authors. Also, the case-control study considered the severity of the head injury and found this factor determined the risk of PD.

1.4.7.2. Anaesthesia

Surgery with general anaesthesia has been suggested as a risk factor for PD. Case reports have described the emergence of parkinsonian symptoms, such as
rigidity following surgery involving general anaesthesia. In one such report, a 54 year old male developed a “dystonic, parkinsonian-type phenomenon” following emergence from general anaesthesia and was diagnosed with PD 18 months later [118]. While the patient did not have any motor symptoms prior to the surgery, he mentioned complaints of persistent anxiety, a lack of a general sense of well being, and recurrent constipation, which are non-motor symptoms often reported in people with PD prior to onset of disease, suggesting that he may have had undiagnosed PD prior to the operation [119]. It is likely that the trauma of surgery or the anaesthetic may act as a trigger for symptoms of underlying PD, rather than being a causative factor.

A follow-up study comparing causes of death between male anaesthesiologists and internists reported an elevated risk of PD for anaesthesiologists compared to internists after more than 10 years follow-up. As both groups were matched on many factors such as smoking and coffee drinking, and the rates were age-standardised, the increased risk may be due to exposure to anaesthetic gases [120]. Most case-control studies have not observed a relationship between PD and treatment with general anaesthetics or surgery [102, 113]. Others report only marginally elevated exposure to general anaesthesia amongst cases [121]. A case-control study comparing history of surgery in Alzheimer’s disease cases, PD cases and non-degenerative neurological control patients reported only a small difference in the number of PD cases reporting surgery in the 5 years before disease onset compared to controls (82.6% versus 76.9%). In the year prior to disease onset, fewer PD cases underwent surgery compared to controls (56.5% versus 63.5%) [121]. As hospital records were used to assess surgical history the results would not be affected by recall bias and any exposure measurement error would be non-differential.
Chloral hydrate, which has been used for many years as a sedative and hypnotic is also a commonly found by-product of water chlorination. It is an intermediate in the synthesis of insecticides and as a metabolite of the hazardous industrial solvent trichloroethylene. Chloral hydrate can form 1-trichloromethyl-1,2,3,4-tetrahydro-b-carboline (TaClo) invivo, a molecule with structural similarities to MPTP, a toxin known to cause parkinsonism [122]. TaClo has been detected in human subjects receiving treatment with chloral hydrate [123]. While TaClo causes cell loss in neuronal and glial cell cultures and induces a slowly developing neurodegenerative process in rats [124], a clear link between chloral hydrate exposure and PD risk is lacking.

1.4.7.3. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

A number of experimental studies indicate a possible therapeutic effect of NSAIDs against progressive dopaminergic neurodegeneration in animal models of PD [125, 126]. Two epidemiological studies have examined the relationship between use of NSAIDs and risk of PD. The Health Professionals Follow-up Study, 1986-2000, and Nurses' Health Study, 1980-1998 prospectively collected data on aspirin and non-aspirin NSAID use. A reduced risk of PD was seen for regular users of aspirin (2 or more tablets per day: RR 0.56, 95% CI 0.26-1.21) and non-aspirin NSAIDs (RR 0.55; 95% CI 0.32-0.96) compared to non-users of NSAIDs, respectively [127].

1.4.8. ENVIRONMENTAL FACTORS

As previously discussed, there is a significant body of evidence to suggest that environmental factors play a role in PD aetiology. Exposure to toxic substances, such as pesticides or neurotoxic metals (e.g. example manganese or lead) could conceivably contribute to mechanisms such as oxidative stress leading to nigral neuronal cell death. If such exposures occur against a backdrop of
genetically determined decreased ability to counteract these toxins, the likelihood of
disease may be greatly increased.

1.4.8.1.MPTP

The occurrence of an irreversible parkinsonian syndrome identical to PD
amongst intravenous drug users who injected drugs contaminated with the chemical
MPTP (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) has been influential in
focusing research into an environmental cause of PD [128, 129]. The toxicological
pathway for MPTP-induced parkinsonism (described previously) has been
identified, providing clues for the search for other possible parkinsonism-inducing
neurotoxins and giving support for an environmental cause of PD [44].

The MPTP model has been highly useful in developing a number of
theories relating to the development of PD. MPTP-susceptibility has been shown in
laboratory mice to be related to age. Older animals suffered neuronal degeneration
with MPTP exposure, while younger animals did not suffer the same degeneration
when administered with twice the dose. Extrapolation of the results to PD in
humans suggests that exposure to environmental toxins throughout life may only
result in PD once changes due to ageing of the nervous system [128] and/or hepatic
detoxification system have occurred [129].

The mode of exposure to MPTP also appears to be important to the
pathophysiology of the resulting syndrome. Chronic delivery of MPTP results in
parkinsonism more closely resembling PD than when acute high doses are
administered. The subpopulations of dopaminergic neurons lost as a result of acute
exposure are not identical to the pattern seen in people with PD [45]. Again,
consideration of the implications of this finding for PD suggests chronic exposure to
environmental toxins, rather than a ‘once off’ exposure, may be influential in the development of the disease.

1.4.8.2. Pesticides

Pesticides include a large number of different chemical compounds that are generally grouped into the categories of insecticides, herbicides, fungicides, rodenticides and fumigants, according to their use. The predominant chemicals used in pesticides have changed substantially over time and greatly increased in diversity.

The herbicide paraquat has been identified as a possible neurotoxic agent due to its molecular structure, which is similar to MPTP [44, 130]. Furthermore, MPP+, a metabolite of MPTP shown to damage neurons in the substantia nigra, has been commercially marketed as a herbicide under the name ‘cyperquat’ [131]. An association between direct exposure to paraquat, cyperquat or diquat and parkinsonian symptoms has been reported in a few cases. Sechi et al. describes a case of a 72-year-old farmer who suffered parkinsonian symptoms including decreased blinking rate, facial masking, flexed posture of arms, trunk, and neck, a walk with short slow steps, postural instability, a slow monotonous voice and cogwheel rigidity after skin contact with 10% diquat 37 dibromide for approximately 10 minutes [132]. The patient's symptoms developed within 10-14 days of the exposure to diquat and did not subside, resulting in persistent parkinsonism. A case report by Meco et al. [133], which describes a 37-year-old man who developed parkinsonism after 3 years of chronic exposure to the fungicide maneb, is also suggestive of a link between the man's fungicide exposure and development of parkinsonian symptoms. Carbon disulfide is used as a solvent in industrial processes and has also been used as a pesticide, usually in the form of a fumigant. Parkinsonism including the symptoms of resting tremor, finger tremor,
cogwheel rigidity, slow speech, and micrographia, has been described in some reports of carbon disulfide exposure [134, 135].

Cases of transient severe parkinsonism following acute organophosphate poisonings have been described [136]. In these reports, the patients developed parkinsonian symptoms such as bradykinesia, facial masking, resting tremor, postural instability and hypophonic speech. Recovery from the parkinsonian symptoms without medication was seen in all patients. While these cases show a link between exposure to pesticides and development of parkinsonian symptoms, the cause of these symptoms is likely to be different to those of PD due to the reversibility of the observed parkinsonian conditions. In these transient cases, organophosphates may have caused a dysfunction in the dopamine receptors of the striatum, rather than damage to the dopamine-producing cells, the characteristic feature of PD. As such, these acute poisoning incidents are likely to be of limited relevance to the aetiology of PD.

1.4.8.3. Neurotoxic metals

Exposure to the neurotoxic metal manganese has been identified as a cause of a parkinsonian syndrome very similar to PD [137, 138]. A feature of manganese intoxication, or manganism, which resembles PD, is the continuation of degeneration long after exposure has ceased [137]. However, there are clinical dissimilarities between manganism and PD, such as less frequent resting tremor, more frequent dystonia, a propensity to fall backward, and failure to achieve a sustained therapeutic response to levodopa [138].

Welding involves joining metals by electric arc or flame with a filler material (or consumable). This process typically produces concentrated particulate fumes and gases containing elements such as manganese, silica, arsenic, nickel,
chromium, beryllium, cadmium, copper, lead, cobalt, zinc, and selenium. Gases released include carbon monoxide, carbon dioxide, ozone, phosgene, and fluorine compounds. The presence of manganese in welding fumes is of particular interest to PD as the neurodegenerative syndrome associated with manganism that includes features of parkinsonism has also been noted to occur in welders [139], as have elevated blood serum levels for manganese [140]. A connection between exposure to welding fumes and PD aetiology is particularly controversial due to legal action in progress by current and former welders seeking compensation from manufacturers of welding materials for parkinsonism, including PD, they allege was caused by their exposure to welding fumes. There are a number of large class actions underway, and the situation has been compared to that of asbestos-related compensation litigation [141].

1.4.8.4. Environmental Toxins and PD

A number of important and highly suggestive findings in relation to PD and environmental toxins have come from laboratory studies. Laboratory experiments have the advantage of studying the relationship between suspected environmental toxins and development of parkinsonian symptoms under controlled conditions, thereby reducing biases and confounding factors. These studies have also been used to demonstrate a biological plausibility behind theories of environmental exposures and PD. However, while providing suggestive evidence of an environmental cause of PD, laboratory results cannot be treated as definitive evidence of an effect due to the vast difference in the laboratory situation and real-life conditions.

Investigation of organochlorine pesticides has featured in a number of laboratory studies of PD [142-144]. Miller et al. [143] found in studies involving injection of mice with the organochlorine pesticide heptachlor and in vitro studies
with the oxidised metabolite heptachlor epoxide, that the pesticide disrupts nigrostriatal dopamine transport. The researchers postulated that this disruption of dopamine transport could increase susceptibility to endogenous and exogenous dopaminergic toxins, providing a plausible explanation of a link between organochlorine pesticide exposure and incidence of PD.

The herbicide paraquat has also received much attention as a possible aetiological agent for PD [145-147]. Experimental research provides evidence that paraquat, or a metabolite of the herbicide can cross the blood-brain barrier to reach the substantia nigra. Systemic injection of mice with paraquat elicited a dose-dependent decrease in substantia nigra dopaminergic neurons, a decline in the striatal dopamine nerve terminal density and a decrease in ambulatory activity, similar to that obtained with injection of MPTP [130]. These results give credibility to the theory that paraquat may be involved in the aetiology of PD, although are not conclusive due to the significant differences between the experimental and human modes of exposure (acute and injection versus chronic and transdermal absorption or inhalation).

The insecticide rotenone (Derris dust) has been shown to cause highly selective degeneration of nigrostriatal dopaminergic neurons in rats treated by chronic systemic infusion with the pesticide [148]. The clinical symptoms exhibited by the treated rats were similar to those of people with PD, such as bradykinesia and rigidity. Furthermore, fibrillar cytoplasmic inclusions containing ubiquitin and alpha-synuclein accumulated in the nigral neurons mimicking a neuropathological feature of PD. The researchers found that rotenone infusion 10 inhibited complex I of the mitochondrial electron transport chain. This finding is of particular interest as complex I is the site inhibited by MPP+, a metabolite of MPTP and has been shown to be reduced in the brain cells and platelets of people with PD [45, 149]. Overall, the study provided some toxicological evidence to link chronic exposure to
a common pesticide (rotenone) to the anatomical, neurochemical, behavioural and neuropathological features of PD.

The results of another study demonstrated *in vitro* that common pesticides including rotenone, dieldrin and paraquat can induce changes in alpha-synuclein and significantly accelerate the rate of formation of alpha-synuclein fibrils [150]. These studies by Betarbet et al. [148] and Uversky et al. [150] demonstrate a link between pesticides and the formation of Lewy body-like structures, a major pathological feature of PD, providing biological plausibility to the involvement of pesticide exposure in PD aetiology. Studies have also reported that primates exposed to carbon disulfide can develop motor disturbances including slowness of movement, un-coordination of movement, plastic and cogwheel rigidity, and resting tremor. However, other non-parkinsonian symptoms, such as action tremor, were also induced and the pathological characteristics were distinct from PD [150].

Manganese ethylene-bis-dithiocarbamate (Mn-EDBC), a major component of the fungicide manebl, has been implicated in PD pathophysiology, both on its own and synergistically with paraquat, in animal studies [148, 151]. Rats treated with Mn-EDBC developed selective reduction of striatal dopamine and dopaminergic neurodegeneration [148]. Rats treated with both paraquat and manebl together experience sustained decreases in motor activity and potentiated effects that appear to target the nigrostriatal dopamine system.

1.4.9. CLINICAL FEATURES OF PARKINSON’S DISEASE

There are four cardinal features of PD that can be grouped under the acronym TRAP: Tremor at rest, Rigidity, Akinesia (or bradykinesia) and Postural instability. In addition, flexed posture and freezing (motor blocks) have been
included among classic features of parkinsonism, with PD as the most common form. Because of the diverse profiles and lifestyles of those affected by PD, motor and non-motor impairments should be evaluated in the context of each patient’s for diagnosis [152]. The most frequent clinical features associated with PD are discussed below.

1.4.9.1. Bradykinesia

Bradykinesia refers to slowness of movement and is the most characteristic clinical feature of PD, although it may also be seen in other disorders, including depression. Bradykinesia is a hallmark of basal ganglia disorders, and it encompasses difficulties with planning, initiating and executing movement and with performing sequential and simultaneous tasks [153]. The initial manifestation is often slowness in performing activities of daily living and slow movement and reaction times [154, 155]. This may include difficulties with tasks requiring fine motor control (e.g., buttoning, using utensils). Other manifestations of bradykinesia include loss of spontaneous movements and gesturing, drooling because of impaired swallowing, [156] monotonic and hypophonic dysarthria, loss of facial expression (hypomimia) and decreased blinking, and reduced arm swing while walking. Given that bradykinesia is one of the most easily recognisable symptoms of PD, it may become apparent before any formal neurological examination. Assessment of bradykinesia usually includes having patients perform rapid, repetitive, alternating movements of the hand (finger taps, hand grips, hand pronation–supination) and heel taps and observing not only slowness but also decrementing amplitude.

In common with other parkinsonian symptoms, bradykinesia is dependent on the emotional state of the patient. For example, immobile patients who become excited may be able to make quick movements such as catching a ball (or may be able to suddenly run if someone screams “fire”). This phenomenon (kinesia
paradoxica) suggests that patients with PD have intact motor programmes but have difficulties accessing them without an external trigger, such as a loud noise, marching music or a visual cue requiring them to step over an obstacle. Although the pathophysiology of bradykinesia has not been well delineated, it is the cardinal PD feature that appears to correlate best with degree of dopamine deficiency [157]. This is supported by the observation of decreased neuronal density in the substantia nigra in elderly patients with parkinsonism regardless of PD diagnosis [158]. In addition, positron emission tomography in patients with PD has demonstrated that the decreased 18F-fluorodopa uptake in the striatum and accumbens caudate complex is proportional to the degree of bradykinesia [161]. It is hypothesised that bradykinesia is the result of a disruption in normal motor cortex activity mediated by reduced dopaminergic function. In a study assessing recordings from single cortical neurons in rats with haloperidol induced bradykinesia, a decrease in firing rates correlated with bradykinesia [160]. Functional neuroimaging studies also suggest impairment in the recruitment of cortical and subcortical systems that regulate kinematic parameters of movement (eg, velocity)[161]. Conversely, recruitment of various premotor areas, such as those responsible for visuomotor control, is increased [161]. Anatomically, the deficit appears to be localised in the putamen and globus pallidus, [169] resulting in a reduction in the muscle force produced at the initiation of movement. Analysis of electromyographic recordings showed that patients with bradykinesia are unable to energise the appropriate muscles to provide enough force to initiate and maintain large fast movements [162]. Because patients with PD have decreased electromyographic activity, [153] they need a series of multiple agonist bursts to accomplish larger movements.

1.4.9.2. Tremor

Rest tremor is the most common and easily recognized symptom of PD. Tremors are unilateral, occur at a frequency between 4 and 6 Hz, and almost always
are prominent in the distal part of an extremity. Hand tremors are described as supination–pronation (“pill-rolling”) tremors that spread from one hand to the other. Rest tremor in patients with PD can also involve the lips, chin, jaw and legs but, unlike essential tremor, rarely involves the neck/head or voice. Thus a patient who presents with head tremor most likely has essential tremor, cervical dystonia, or both, rather than PD. Characteristically, rest tremor disappears with action and during sleep. Some patients also report an “‘internal’” shaking that is not associated with a visible tremor [163]. The tremor of PD is differentiated from that of essential tremor by a number of features. Some patients with PD have a history of postural tremor, phenomenologically identical to essential tremor, for many years or decades before the onset of parkinsonian tremor or other PD related features. In addition to rest tremor, many patients with PD also have postural tremor that is more prominent and disabling than rest tremor and may be the first manifestation of the disease [164, 165]. Parkinson’s related postural tremor (“re-emergent tremor”) is differentiated from essential tremor in that the appearance of tremor is often delayed after the patient assumes an outstretched horizontal position [164]. Because re-emergent tremor occurs at the same frequency as classical rest tremor and is responsive to dopaminergic therapy, it is likely that it represents a variant of the more typical rest tremor. There are several clues to the diagnosis of existent essential tremor when it coexists with PD, including longstanding history of action tremor, family history of tremor, head and voice tremor, and no latency when arms are outstretched in a horizontal position in front of the body, although some patients may also have a re-emergent tremor related to their PD, tremulous handwriting and spiral, and improvement of the tremor with alcohol and beta-blockers. The occurrence of rest tremor is variable among patients and during the course of the disease. In one study, Hughes and colleagues [166] reported that 69% of patients with PD had rest tremor at disease onset and that 75% had tremor during the course of their disease. Tremor was lost in 9% of patients late in the disease. Others have reported that a small proportion of patients (11%) never have tremor, [167]
although a prospective study in patients with autopsy proven disease found that 100% of patients had tremor at some point [168]. Clinical–pathological studies have demonstrated that patients with PD and prominent tremor have degeneration of a subgroup of midbrain neurons, whereas this area is spared in PD patients without tremor.

1.4.9.3. Rigidity

Rigidity is characterised by increased resistance, usually accompanied by the “cogwheel” phenomenon, particularly when associated with an underlying tremor, present throughout the range of passive movement of a limb (flexion, extension or rotation about a joint). It may occur proximally (eg, neck, shoulders, hips) and distally (eg, wrists, ankles). Reinforcing manoeuvres (eg, voluntary movements of the contralateral limb), known as the Froment’s manoeuvre, [169] usually increase rigidity and are particularly useful in detecting mild cases of rigidity. Rigidity may be associated with pain, and painful shoulder is one of the most frequent initial manifestations of PD although it is commonly misdiagnosed as arthritis, bursitis or rotator cuff injury [170, 171]. A prospective study of 6038 persons (mean age 68.5 years) with no evidence of dementia or parkinsonism at baseline found that the presence of stiffness, tremor and imbalance were each associated with increased risk for PD (hazard ratios 2.11, 2.09 and 3.47, respectively) [172]. Among this cohort, 56 new cases of PD were identified over a mean followup of 5.8 years.

1.4.9.4. Postural deformities

In addition, rigidity of the neck and trunk (axial rigidity) may occur, resulting in abnormal axial postures (eg, anterocollis, scoliosis). Postural deformities resulting in flexed neck and trunk posture and flexed elbows and knees are often associated with rigidity. However, flexed posture generally occurs late in
the disease. Striatal limb deformities (eg, striatal hand, striatal toe) may also develop in some patients. Striatal hand is characterised by ulnar deviation of the hands, flexion of the metacarpophalangeal joints and extension of the proximal and flexion of the distal interphalangeal joints. Striatal foot is characterised by extension or flexion of the toes [173, 174]. In one study, striatal toe (extension of the big toe) was reported in 21% of patients with clinically diagnosed PD [175]. Patients with striatal deformities tend to be younger and to experience earlier onset of initial parkinsonian symptoms. Other skeletal abnormalities include extreme neck flexion (‘dropped head’ or ‘bent spine’), truncal flexion (camptocormia) and scoliosis [176, 177]. Camptocormia is characterised by extreme flexion of the thoracolumbar spine. The condition is exacerbated by walking and is relieved by sitting, lying in the supine position or by volitionally extending the trunk when the patient leans against a wall or a high walker or a table [177]. In addition to PD, other causes of camptocormia include dystonia and extensor truncal myopathy.[178]. Another truncal deformity is the Pisa syndrome, which is characterised by a tilting of the trunk, particularly when sitting or standing [179].

1.4.9.5. Postural instability

Postural instability due to loss of postural reflexes is generally a manifestation of the late stages of PD and usually occurs after the onset of other clinical features. The pull test, in which the patient is quickly pulled backward or forward by the shoulders, is used to assess the degree of retropulsion or propulsion, respectively. Taking more than two steps backwards or the absence of any postural response indicates an abnormal postural response. Postural instability (along with freezing of gait) is the most common cause of falls and contributes significantly to the risk of hip fractures [180]. The long latency to the onset of falls differentiates PD from other neurodegenerative disorders, such as progressive supranuclear palsy (PSP) and multiple systems atrophy (MSA) [181]. In one study, the average time
from onset of symptoms to the first fall was 108 months in patients with PD compared with 16.8 and 42 months, respectively, in patients with PSP and MSA [180].

Several other factors also influence the occurrence of postural instability in patients with PD. These include other parkinsonian symptoms, orthostatic hypotension, age related sensory changes and the ability to integrate visual, vestibular and proprioceptive sensory input (kinesthesia) [182, 183]. The fear of falling can further impair balance control in patients with PD [184]. In one study, 38% of those evaluated experienced falls, and 13% fell more than once a week [185]. As expected, the frequency of falls correlated with the severity of disease [185]. Treatment (dopaminergic therapy, pallidotomy, deep brain stimulation) can improve some axial signs [186] but usually does not robustly improve postural instability, measured by platform tilt and visual tilt [187]. Targeting other nuclei for deep brain stimulation in addition to the subthalamic nucleus and globus pallidus, such as the zona incerta and pedunculopontine nucleus, is being explored as a potential surgical treatment of gait difficulties and postural stability [188].

1.4.9.6. Freezing

Freezing, also referred as motor blocks, a form of akinesia (loss of movement) and is one of the most disabling symptoms of PD [189]. Although freezing is a characteristic feature of PD, it does not occur universally [190]. Based on responses by 6620 patients to a questionnaire sent to 12 000 members of the German Parkinson Association, 47% of patients reported freezing, it occurs more frequently in men than in women and less frequently in patients whose main symptom is tremor [191]. Freezing most commonly affects the legs during walking, but the arms and eyelids can also be involved [192]. It typically manifests as a sudden and transient (usually, 10 s) inability to move. This may include hesitation
when beginning to walk (start hesitation) or a sudden inability to move the feet during specific situations (eg, turning or walking through a narrow passage, crossing busy streets, approaching a destination). Freezing is associated with substantial social and clinical consequences for patients. In particular, it is a common cause of falls [190]. Five subtypes of freezing have been described: start hesitation, turn hesitation, hesitation in tight quarters, destination hesitation and open space hesitation [193]. Episodes are more severe in the OFF state and are mitigated by levodopa therapy. In addition, patients often develop tricks to overcome freezing attacks. This includes marching to command, stepping over objects (eg, a walking stick, cracks in the floor), walking to music or a beat, and shifting body weight [194, 195]. Risk factors for the development of freezing include the presence of rigidity, bradykinesia, postural instability and longer disease duration [189]. In contrast, tremor at disease onset is associated with a decreased risk of freezing. As freezing typically occurs later in the course of the disease or is not the predominant symptom, alternative diagnoses should be considered when these presentations occur. Freezing, particularly when it occurs during the ON period, does not usually respond to dopaminergic therapy, but patients treated with selegiline have been found to be at lower risk [196]. Botulinum toxin injections, although effective for a variety of parkinsonian symptoms such as tremors, dystonia and sialorrhea, have not been found consistently effective in the treatment of freezing [197].

1.4.9.7. Non-Motor features

Non-motor symptoms are a common and under appreciated feature of PD [198]. These include autonomic dysfunction, cognitive/ neurobehavioral disorders, sleep and sensory abnormalities.
1.4.9.8. Autonomic dysfunction

Autonomic failure may be the presenting feature of PD, although it is more typically associated with MSA. Features include orthostatic hypotension, sweating dysfunction [199], sphincter dysfunction and erectile dysfunction [200, 201]. A community based study found that 47% (42/89) of PD patients met the diagnostic criteria for orthostatic hypotension [202].

1.4.9.9. Cognitive and neurobehavioural abnormalities

Neuropsychiatric disturbances can be as disabling as motor symptoms. The Sydney Multicenter Study of PD found that 84% of patients evaluated showed cognitive decline and that 48% met the diagnostic criteria for dementia after 15 years of follow-up [203]. Another community based prospective study found that patients with PD are at almost sixfold increased risk for dementia [204]. PD related dementia is also associated with a number of other neuropsychiatric comorbidities. Among 537 such patients, depression (58%), apathy (54%), anxiety (49%) and hallucinations (44%) were frequently reported [205]. In a study of 114 patients with PD, 27.6% screened positive for depression during the average 14.6 months of follow-up; 40% were neither treated with antidepressants nor referred for further psychiatric evaluation [206]. In addition to cognitive and affective disorders, many patients with PD exhibit features of obsessive–compulsive and impulsive behaviour, such as craving (especially for sweets) [207], binge eating, compulsive foraging, hypersexuality, pathological gambling, compulsive shopping and punding, characterised by intense fascination with repetitive handling, examining, sorting and arranging of objects [208]. These behavioural symptoms, sometimes referred to as “hedonistic homeostatic dysregulation”, have been attributed to dopamine dysregulation syndrome associated with the use of dopaminergic drugs, particularly dopamine agonists, but the mechanism of these aberrant behaviours is not well understood [209].
1.4.9.10. Sleep disorders

Although sleep disturbances (eg, excessive sleepiness, sleep attacks) were once largely attributed to the pharmacological therapy for PD [210], some clinicians now believe that these features are an integral part of the disease [211]. This is supported by the observation that rapid eye movement sleep behaviour disorder, which occurs in approximately one-third of patients with PD, is a substantial risk factor for the development of PD [212-215]. Rapid eye movement sleep behaviour disorder, now considered a pre-parkinsonian state, is characterised by an increase in violent dream content [215] accompanied by talking, yelling, swearing, grabbing, punching, kicking, jumping and other dramatic, violent and potentially injurious motor activity, which may also involve the bed partner. Insomnia, particularly sleep fragmentation, is also frequent (50% prevalence), but the occurrence is highly variable among patients [216, 217]. The sleep abnormalities observed in patients with PD may possibly be related to a 50% loss of hypocretin (orexin) neurons [218, 219]. Although excessive daytime sleepiness may contribute to fatigue, this common symptom is also seen independently of sleepiness [220].

1.4.9.11. Sensory abnormalities

Sensory symptoms such as olfactory dysfunction, pain, paresthesia, akathisia, oral pain and genital pain are frequent but are often not recognized as parkinsonian symptoms [221-223]. One study found that olfactory dysfunction (hyposmia) may be an early marker of PD it correlated with a 10% increased risk for the disease 2 years later compared with other asymptomatic relatives [224]. A study involving 62 pairs of twins discordant for PD found that smell identification was reduced in twins affected with PD than in those who were asymptomatic [225]. It has been postulated that olfactory dysfunction is related to either neuronal loss in
the corticomedial amygdala [226] or to decreased dopaminergic neurons in the olfactory bulb.

1.4.10. DIAGNOSIS OF PARKINSON’S DISEASE

The diagnosis of PD is straightforward when cardinal clinical signs and symptoms as bradykinesia, rigidity, and resting tremor are present. However, these main features of PD are shared, at least in part, by essential tremor (ET), multisystem atrophy (MSA), progressive supranuclear palsy (PSP), vascular parkinsonism (VP), dementia with Lewy bodies, corticobasal degeneration, Alzheimer's disease, and drug-induced parkinsonism. Besides delineating PD from the above parkinsonian disorders, distinguishing PD from normality can also be difficult, especially in early stage of the disease [227].

The gold standard for the diagnosis of PD is post-mortem neuropathological examination [228]. Neuropathological studies show that even at end-stage disease the clinical diagnostic accuracy for PD varies between 75–90%, with MSA and PSP accounting for most false positives [229-231]. Diagnostic accuracy is certainly less than 90% in earlier disease, as Litvan et al. found that the median sensitivity for the diagnosis of PD increased from 73% at the first visit to 80% to the last visit after a mean follow-up of 9 years, and the median positive predictive value increased from 46 to 64% [232].

A reliable test to diagnose PD is important for two reasons. Prognosis and management of PD and other parkinsonian disorders differ considerably [223]. Several procedures have been proposed to diagnose PD, olfactory and neuropsychological tests, functional imaging with Positron Emission Tomography (PET) or Single Photon Emission Computer Tomography (SPECT), transcranial sonography, biomarkers and DNA tests [234-237]. At the moment neuroimaging
techniques such as positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI) and transcranial sonography have increasingly been employed to detect PD, to elucidate the neuropathological mechanisms and compensatory responses underlying symptoms and treatment associated complications, and to monitor disease progression in vivo. However, the usage of these imaging technique in routine clinical practice is limited by high costs and a relative short half-life of its radioactive tracers [238-241].

1.4.10.1. PET and SPECT

PET and SPECT have been extensively employed to elucidate the functional changes associated with PD and other neurodegenerative parkinsonian disorders. Both modalities provide a means of assessing: (1) disease severity as reflected by presynaptic dopamine terminal dysfunction, (2) subclinical dysfunction in subjects who are at risk for PD, (3) disease progression and the effects on this of putative neuroprotective agents, and (4) changes in non-dopaminergic neurotransmission. Additionally, PET studies with the peripheral benzodiazepine ligand ¹¹C-(R)-PK11195, a selective marker of activated microglia [242] have been used for imaging brain inflammation in vivo in PD patients and to help clarify the role of activated microglia in the ongoing degenerative process.

Assessment of the functional integrity of presynaptic nigrostriatal projections is a major goal for the functional imaging techniques used in the evaluation of patients with parkinsonian features.
1.4.10.1.1. Measurement of striatal aromatic amino acid

Decarboxylase activity $^{18}$F-dopa PET was the first neuroimaging technique validated for the assessment of presynaptic dopaminergic integrity. The uptake of $^{18}$F-dopa in the striatal nuclei over 90 min, as measured by an influx constant Ki, reflects both the density of the axonal terminal plexus and the activity of the striatal aromatic amino acid decarboxylase (AADC), the enzyme responsible for the conversion of $^{18}$F-dopa to $^{18}$F-dopamine. Measurements of $^{18}$F-dopa uptake in the striatum of patients with PD will, therefore, be influenced by the number of remaining dopaminergic cells. This is supported by pathological studies which have demonstrated that levels of striatal $^{18}$F-dopa uptake correlated well with nigral cell counts in both human cases and in non-human primates where parkinsonism was induced by the nigral toxin MPTP [243, 244]. However, particularly in early stages of disease, $^{18}$F-dopa PET may underestimate the degenerative process due to the presence of compensatory upregulation of AADC in remaining terminals [245].

Putamen uptake of $^{18}$F-dopa in PD has been shown to correlate with the clinical severity of locomotor disability as measured by the UPDRS [246-248]. Interestingly, while putamen $^{18}$F-dopa reductions correlate well with the degree of rigidity and bradykinesia in PD this is not true of tremor severity suggesting that either non-nigrostriatal and/or non-dopaminergic pathways are implicated in the pathogenesis of this symptom. Typically, PD patients show a gradient of reduced striatal $^{18}$F-dopa uptake along a rostro-caudal axis. Patients with hemiparkinsonism have their greatest $^{18}$F-dopa uptake reduction in the dorsal posterior putamen contralateral to the side of clinical symptoms [249]. As the disease progresses to become bilateral, additional reductions are seen within the ventral and anterior putamen and dorsal caudate. In the most advanced stages, uptake within the ventral head of caudate also falls. These $^{18}$F-dopa PET findings are in line with post-mortem data that have reported an uneven pattern of dopamine loss in the striatum
in PD, posterior dorsal putamen being targeted. Nigral cell counts are lowest in ventrolateral subregions which send dopaminergic projections to the dorsal putamen. [250]. Reductions of $^{18}$F-dopa uptake in PD can be localised at a voxel level across the whole brain by interrogating PET images with statistical parametric mapping (SPM). This analytical approach has made it possible to detect changes in $^{18}$F-dopa uptake in extrastriatal as well as striatal regions and to explore compensatory responses to the neurodegenerative process at different stages of the disease. With SPM, several authors have reported increases in $^{18}$F-dopa uptake in dorsolateral prefrontal cortex, anterior cingulate, and globus pallidus interna of patients with early PD compared to both normal controls and patients with more advanced disease [251-253]. It is likely that these increases in extrastriatal $^{18}$F-dopa uptake in early PD reflect compensatory upregulation of AADC though some uptake of $^{18}$F-dopa into serotonergic terminals may also be a contributor. Whone et al. observed a 40% increase in $^{18}$F-dopa uptake in the globus pallidus interna in early PD which was then lost in advanced disease as motor fluctuations developed [253]. It may well be that raised pallidal dopamine storage in early PD is important for normalising basal ganglia output to the ventral thalamus and motor cortex and, when pallidal as well as putamen dopamine storage fails, motor responses to levodopa therapy become fluctuating and unpredictable.

1.4.10.1.2. Measurement of presynaptic dopamine transporter binding

The presynaptic dopamine transporter (DAT) is the plasma membrane transporter responsible for the high-affinity uptake of dopamine. It is found exclusively in dendrites and axons of dopaminergic neurons and is therefore a potential marker of integrity of nigrostriatal projections. Several PET ligands ($^{11}$C-CFT, $^{18}$F-CFT, $^{18}$F-FP-CIT, and $^{11}$C-RTI-32) and SPECT tracers (such as $^{123}$I-β-CIT, $^{123}$I-FP-CIT, $^{123}$I-altropane, $^{11}$C-methylphenidate, and $^{99}$mTc-TRODAT-1) are now available to measure DAT availability. $^{123}$I-β-CIT, a tropane derivative, binds with
equal nanomolar affinity to DAT, noradrenergic (NART), and serotonergic (SERT) transporters. Striatal uptake at 24 h post-injection primarily reflects DAT binding whereas brainstem uptake at 1 h post-injection reflects SERT binding. Similarly to $^{18}$F-dopa, striatal $^{123}$I-β-CIT uptake correlates well with stage of disease and symptom severity in PD, particularly with bradykinesia but not rest tremor [254-256]. A disadvantage of $^{123}$I-β-CIT is its slow striatal uptake kinetics. It takes 24 h to equilibrate in this brain region following its administration so SPECT must be delayed to the following day. More recently developed SPECT tracers such as $^{123}$I-FP-CIT, $^{123}$I-altropane, and $^{123}$I-PE21, have faster uptake kinetics though they give higher non-specific signals. In practice diagnostic scans can be performed within 2 to 3 h of tracer administration. $^{99m}$Tc-TRODAT-1 has the advantage of being technetium based and so available in kit form. Its specific signal, however, is lower than the $^{123}$I based SPECT tracers. In general, all these DAT markers show similar findings in PD to those seen with $^{18}$F-dopa PET and are able to differentiate early PD from normal subjects with a sensitivity of around 90%. In contrast to $^{18}$F-dopa, striatal uptake of DAT ligands in early PD may overestimate the reduction in terminal density due to the relative downregulation of DAT in remaining neurons as a response to nigral neuron loss in order to maintain synaptic dopamine levels. While striatal $^{18}$F-dopa does not appear to be age dependent in healthy subjects, DAT binding falls with age [257-259].

1.4.10.1.3. Measurement of the vesicular monoamine transporter 2

The type-2 vesicular monoamine transporter (VMAT2) is exclusively expressed in the brain and is responsible for the uptake of monoamines from the cytoplasm into the secretory vesicles in dopamine neurons [260]. $^{11}$C-dihydrotetrabenazine (DTBZ) is a PET tracer that binds to VMAT2. Lee et al. compared striatal uptake of $^{11}$C-dihydrotetrabenazine, $^{18}$F-dopa, and the DAT ligand $^{11}$C-methylphenidate in PD. They found that $^{18}$F-dopa Ki was reduced relatively less
than the $^{11}$C-dihydrotetrabenazine binding potential in the parkinsonian striatum, while $^{11}$C-dihydrotetrabenazine binding was reduced less than $^{11}$Cmethylphenidate binding [261]. This finding is in line with the presence of relative AADC upregulation and DAT downregulation in the striatum of parkinsonian patients in order to increase dopamine turnover and diminish its re-uptake. The authors propose that $^{11}$C-dihydrotetrabenazine PET gives the most reliable measurement of the density of dopaminergic terminals. This suggestion, however, remains to be validated by comparing $^{11}$C-dihydrotetrabenazine striatal binding with post-mortem nigral cell counts and demonstrating that dopaminergic drugs have no effect on tracer uptake.

1.4.10.1.4. Detection of subclinical disease

Measures of dopaminergic presynaptic integrity with both $^{18}$F-dopa PET and $^{125}$I-β-CIT SPECT have allowed detection of subclinical dysfunction in subjects who are at risk for PD. Reductions in $^{18}$F-dopa putaminal uptake has been reported in 18% of asymptomatic dizygotic co-twins and in 55% of asymptomatic monozygotic co-twins of patients with idiopathic PD. Over the 4-year follow-up of this study, two of ten asymptomatic monozygotic co-twins subsequently developed clinical parkinsonism and all 10 subjects showed a further decrease in putaminal $^{18}$F-dopa uptake [262]. A significant reduction of putaminal $^{18}$F-dopa uptake has also been reported in around 25% of asymptomatic siblings in kindreds with familial PD, and about one-third of those with abnormal imaging developed clinical parkinsonism over a five-year follow-up [263]. These findings support a role of inheritance in PD, but do not fully rule out the effect of possible concomitant environmental factors.

PARK2 is a recessive form of Parkinson's disease caused by parkin gene mutations. Recently, two separate studies [264, 265] have reported reductions of
striatal $^{18}$F-dopa uptake in asymptomatic carriers of a single parkin mutation compared to normal subjects. Longitudinal studies are now required to establish whether these subjects will convert to clinical PD later on in life. $^{123}$I-β-CIT SPECT has been used to evaluate dopamine terminal integrity in relatives of PD patients with no parkinsonian symptoms but with a complaint of idiopathic hyposmia, a known risk factor for PD. 11% of the relatives exhibited hyposmia on UPSIT testing and 17.5% (7 out of 40) of these siblings with hyposmia had reduced striatal $^{123}$I-β-CIT binding. 57% (4 out of 7) of those hyposmic relatives with subclinically reduced DAT binding converted to clinical PD over a 2-year follow-up [266]. While, $^{123}$I-β-CIT SPECT can detect subclinical dysfunction in PD relatives only 2% in total, however, exhibit both hyposmia and dopaminergic loss.

1.4.10.1.5. Disease progression and the effects of putative

PET and SPECT have been used to monitor the progression of nigrostriatal degeneration in PD. Several series have now demonstrated that the loss of striatal $^{18}$F-dopa uptake occurs more rapidly in PD patients than in age-matched controls [267-270]. In these studies the mean annual rate of $^{18}$F-dopa uptake decline in PD patients has been reported to range from 8% to 12% in the putamen and 4% to 6% in the caudate, whereas the annual decline in normal volunteers is lower than 1% (0.5% and 0.7% in the putamen and in the caudate respectively) [270]. It has been suggested that the absolute rate of decline does not vary between different regions of the striatum. In a 5-year longitudinal study with $^{18}$F-dopa PET, Nurmi et al. evaluated the annual rate of decline of tracer uptake in different striatal subregions [270]. They found a 10.3% annual reduction in $^{18}$F-dopa uptake in the posterior putamen and an 8.3% reduction in the anterior putamen. Caudate nucleus showed a 5.9% annual reduction. The absolute rate of decline however was similar in all striatal subregions. An analogous study was performed in 31 untreated patients with early PD [271]. Patients were studied with $^{18}$F-dopa PET twice, at the
time of the diagnosis and 2 years later. Results from this study also indicate a similar rate of progression between the subregions of the striatum. Based on this evidence, $^{18}$F-dopa PET provides a reliable biological marker of the progression of PD. It has been argued, however, that decreases in striatal $^{18}$F-dopa uptake over time may not provide an accurate measure of the neurodegenerative process as they reflect both neuronal loss and failure of compensatory mechanisms (AADC upregulation).

$^{123}$I-β-CIT, $^{123}$I-FP-CIT, and $^{123}$I-IPT SPECT and $^{18}$F-CFT PET have all been used to monitor the rate of the loss of DAT binding in PD. Several $^{123}$I-β-CIT SPECT studies have evaluated the rate of PD progression and reported similar rates of the loss of putamen dopamine transporters with a mean 8% annual decline [257, 272-274]. A confounding factor when assessing disease progression with $^{123}$I-β-CIT SPECT is that tracer uptake decreases with age (3.3% to 10% per decade) in healthy subjects [257-259]. Nurmi et al. [275] have used 18F-CFT PET to investigate DAT loss in striatal subregions in patients with early PD. At variance with previous $^{18}$F-dopa PET findings, they found that the decline in tracer uptake was significantly different in anterior and posterior putamen. When the rates of progression were calculated compared to the normal control mean, the caudate had the highest rate of progression (5.6%), followed by the anterior putamen (5.3%) and then the posterior putamen (3.3%). Additionally, the absolute decline in $^{18}$F-CFT PET uptake was greater in the less affected putamen. If confirmed in larger longitudinal studies, this finding would suggest that progression is nonlinear – possibly exponential – and slower in the posterior putamen where the disease is more advanced at baseline.

At present, $^{11}$C-DTBZ PET is only available in few centres and not many longitudinal studies have been performed with this technique. The Vancouver group has reported two separate longitudinal studies in PD patients over a 4-year follow-up [276, 277]. The annual rate of decline in putamen DTBZ was around 5% of
baseline. A faster rate of progression was observed in patients with a milder disease. Due to their capacity to monitor the loss of dopaminergic function in PD objectively and to their relatively wide availability worldwide, $^{18}$F-dopa PET and $^{123}$I-β-CIT SPECT have been used as biomarkers of disease progression when assessing the efficacy of putative neuroprotective agents.

1.4.10.2. Magnetic resonance imaging

MRI is far more widely available than PET and SPECT and is most commonly used in clinical practice to differentiate idiopathic PD from secondary causes of parkinsonism, such as vascular disease and other structural lesions. MRI findings may also help differentiate PD from multiple system atrophy by showing a reduced T2-weighted Putman signal, and progressive supranuclear palsy and cortical-basal degeneration by revealing midbrain and cortical atrophy [278]. Conventional MRI is normal in patients with idiopathic PD without dementia, as standard MRI sequences have proved unable to detect definitive abnormalities in the basal ganglia structures. Several researchers have used MRI sequences designed to reveal changes in midbrain iron content as post-mortem studies in PD have shown an increase in iron concentration in the SN [279]. Early MRI studies failed to show significant differences in SN iron levels between PD patients and controls [280, 281]. New MRI methodologies, however, appear to be more sensitive to iron increases in PD patients [457–460]. Using inversion recovery white and grey matter signal-suppression sequences, structural changes have been found in the SN of PD patients, even at very early stages, with significant differences between patients and control group [282, 283]. In one of these studies, MRI changes within the SN of PD patients correlated with a striatal dopaminergic function measured by $^{18}$F-dopa PET. $^{18}$F-dopa PET, however, it is more reliable than inversion recovery MRI in discriminating patients with moderately severe PD from normal subjects [283]. Recently, Martin et al. [285] have assessed 26 untreated PD patients with a 3 T MRI
and a multiple gradient echo sequence designed for rapid single-scan mapping of the proton transverse relaxation rate (R2*). They found that PD patients had significantly higher R2* values in the lateral SNc compared to controls. Interestingly, there was an association between the lateralized motor score from the clinically most affected side and R2* values obtained from the opposite lateral SNc. Longitudinal studies are currently underway to assess the validity of nigral R2* measurements as a marker of disease duration. Voxel based morphometry (VBM) [286] is an MRI technique that localises significant changes in grey matter density related to disease.

MR images are spatially normalized into standard stereotaxic space, segmented into gray and white matter and cerebrospinal fluid, smoothed and submitted to statistical parametric mapping at a voxel level. VBM has been used in PD to evaluate patterns of brain atrophy in patients with and without dementia [287-291]. These studies have revealed significant cortical atrophy in PD patients with dementia which progresses over time. Feldmann et al. [292] have recently reported VBM findings in a group of PD patients with and without depression. They found gray matter decrease in the bilateral orbitofrontal cortex, the right superior temporal pole, and the limbic system of depressed PD patients.

Diffusion tensor imaging (DTI) and diffusion tensor tractography (DTT) are promising new MRI techniques which evaluate the integrity of tracts in white matter and, indirectly, neuronal connectivity in the brain. Normally water diffusion is constrained along nerve fibres in brain tissue and so is anisotropic. Degeneration of tracts leads to the loss of this directionality of diffusion or anisotropy. DTI measures the direction and magnitude of diffusivity of water molecules in tissues and can be used as an index of damage to neuronal tracts. There are two main quantities of interest, the mean apparent diffusion coefficient (ADC), which measures total molecular motion averaged over all directions, and fractional
anisotropy (FA), which is a measure of the directional diffusivity of water. DTT is a computational procedure that reconstructs major fiber bundles in the brain based on the anisotropy of water movement in myelinated white matter. Yoshikawa et al. [293] and Chan et al. [294] have reported lower values of FA in the SN of PD patients compared to controls. FA values were inversely correlated with disease severity [294]. Another study has shown evidence of olfactory tract degeneration in early PD patients [295]. This finding is in line with the 50% prevalence of hyposmia in PD on UPSIT testing. Finally, DTI has been used to investigate non-motor symptoms of PD. Matsui et al. scanned a group of PD patients with and without depression and found bilateral abnormalities in the anterior cingulate in depressed patients [296]. The same group also compared PD patients with and without dementia and found abnormalities in the posterior cingulate in the latter [297]. These interesting findings remain to be confirmed by larger studies.

1.4.10.3. Transcranial sonography

Transcranial brain sonography (TCS) is a neuroimaging technique, which measures brain tissue echogenicity through the intact skull. TCS is usually performed using a phase-array ultrasound system with a 2.5 MHz transducer. The butterfly-shaped mesencephalon can be identified through a preauricular acoustic bone window in most subjects. The typical TCS finding in PD patients is an increased echogenicity from the lateral midbrain, probably arising from the SN and reflecting increased amounts of iron deposition. The SN, which in normal subjects appear as a small patchy area of slightly increased echogenicity, become more demarcated and identifiable in PD patients. SN hyperechogenicity has been reported in up to 90% of clinically probable PD patients assessed with this technique [298 - 300]. A similar finding, however, has also been reported in 17% of patients with essential tremor [301], 40% of depressed patients without signs of PD [302], and in 10% of healthy age-matched volunteers where it could possibly reflect subclinical
involvement of the nigrostriatal system [303, 304]. Conversely, normal SN echogenicity is observed in atypical parkinsonian syndromes despite involvement of the dopaminergic system and this may help in the differential diagnosis with PD [305]. Levels of nigral echogenicity in PD do not correlate with striatal dopamine transporter binding measured with $^{123}$I-FP-CIT SPECT [306] and do not appear to change over a five-year follow-up period despite clinical progression [307]. This suggests that SN hyperechogenicity is a trait rather than state marker of PD, probably reflecting disturbances of iron metabolism rather than neuronal degeneration.

1.4.11. BIOMARKERS

Biomarkers are objectively measured characteristics that are indicators of normal biological processes, pathogenic processes, or responses to interventions.

1.4.11.1. CLINICAL BIOMARKERS

Diagnosis of PD in many cases is straightforward. A patient may present with asymmetric resting tremor and bradykinesia with associated features of mild facial masking and reduced arm swing on the side with the tremor. If the patient progresses gradually and responds well to levodopa over time, then we can be quite certain ($\geq 90\%$) that the patient will have PD pathology at autopsy [308].

Unfortunately, many patients with parkinsonism do not have classic features at presentation that made difficulty in diagnosis [309]. In primary care, patients may be labeled as having PD when in fact they have tremor, ET, or AP up to 50% of the time [310]. Even in the hands of movement disorder specialists, perhaps 10% to 15% of patients with “early PD” are misdiagnosed [311].
1.4.11.2. Idiopathic rapid eye movement behavior disorder

Rapid eye movement (REM) behavior disorder (RBD) is characterized by an absence of atonia during REM sleep. It may be idiopathic or be caused by a variety of conditions, such as withdrawal from sedative–hypnotics or other drugs. The diagnosis requires a polysomnogram with chin electromyography (EMG), which typically shows increased tonic chin EMG activity during REM sleep. Idiopathic RBD (iRBD) is perhaps one of the earliest signs of PD in many patients, often preceding the motor symptoms and signs of PD by many years [312, 313]. Recent research has identified iRBD as one of the premotor symptoms/signs most correlated with future development of a synucleinopathy MSA, dementia with Lewy bodies or PD or dementia, with a 12-year risk of 52.4% for developing one of these conditions [312]. Given these patients’ high risk for developing a synucleinopathy, use of this clinical biomarker will help clinicians and researchers test potential disease modifying therapies earlier than ever before in the premotor phase of PD. There appears to be a definite correlation between iRBD and neuroimaging findings typical of PD on transcranial ultrasound (TCUS) and olfactory deficits, further indicating that iRBD is an excellent premotor marker of future neurodegenerative disease [314].

1.4.11.3. Olfactory deficits

Olfactory identification deficits may precede the motor features of PD by years [315]. Olfactory identification deficits on the University of Pennsylvania Smell Identification Test (UPSIT) or other tests (Sniffin’ Sticks; Heinrich Burghart, Wedel, Germany) may be useful in differentiating PD from other movement disorders and other forms of parkinsonism, but this remains to be validated in clinical practice [316]. In contrast to many other potential biomarkers, olfaction is impaired very early in the disease course of PD, even before motor symptoms begin [315, 316]. Olfactory deficits occur in 70% to 100% of PD patients, and these
deficits are not correlated with disease duration, are bilateral, and importantly, are not influenced by levodopa treatment [317]. Many, if not most, PD patients are unaware of their olfactory deficit at presentation [316, 317]. Although olfactory deficits on the UPSIT are not specific to PD, the presence of olfactory deficits in patients in the proper clinical setting may serve as a potential adjunctive biomarker to aid clinicians in the differential diagnosis of PD. Olfactory identification deficits may help differentiate PD from other forms of parkinsonism. Patients with psychogenic parkinsonism, MSA, PSP, corticobasal degeneration (CBD), or vascular parkinsonism [317, 318] do not have significant olfactory deficits relative to patients with PD. In one study using the UPSIT, Wenning et al. [319] found that an UPSIT score of 25/40 had a 77% sensitivity and an 85% specificity for differentiating PD patients from patients with atypical forms of parkinsonism (CBD, MSA, PSP). Receiver operating characteristic curves have been developed for patients based on their sensitivity and specificity estimates for three age groups (≤60, 61–70, and ≥71 years) [316]. Sensitivity estimates for PD using University of Pennsylvania Smell Identification Test (UPSIT) are in the range of 76% to 91%, depending on the age and the gender of the tested patient [316]. Patients with the more akinetic-rigid form of PD many have slightly more olfactory dysfunction on average than patients with the tremor-dominant form, and men appear to have greater impairment in sense of smell in PD relative to women [316].

In early PD, olfactory deficits may be present more often than tremor. Tremor, the most common presenting complaint for PD patients, occurs in 75% to 80% of patients. Given that olfactory deficits can precede the onset of motor symptoms in PD by many years, impaired olfaction may be an inexpensive, widely available biomarker for early detection and diagnosis of PD, especially when coupled with neuroimaging and/or clinical examination.
1.4.11.4. Constipation

There are many ways to define and characterize constipation, including bowel movement frequency and stool consistency. Constipation is very common in the general population, and very common in PD, affecting most patients. It may precede the development of PD by many years, and in the Honolulu Aging Study, bowel movement frequency was inversely correlated with risk of future development of PD, with those having the fewest bowel movements having the greatest risk of developing PD. Constipation in isolation would be quite nonspecific as an early biomarker of PD but in combination with olfactory deficits and/or neuroimaging markers, suggested to be useful in diagnosis.

1.4.11.5. Genetic biomarkers

There are numerous genetic factors that may play a role in susceptibility to or lead to development of PD; however, the vast majority of PD cases (≥85%) in most clinical settings are sporadic and not inherited. There are certain genetic mutations that bear mentioning here given their more prominent role. In patients with juvenile-onset PD (PD with an age of onset <20 years), one should certainly consider commercially available testing for PARK2 or parkin, given that up to 75% of patients with juvenile parkinsonism may have this autosomal recessive form of PD. Likewise, consideration of the leucine-rich repeat kinase-2 (LRRK2) mutations (especially G2019S) in autosomal dominant parkinsonism would be wise, given that this has emerged as the most common genetic form of PD in Caucasians and also is found in 20% to 40% of Ashkenazi Jews and North African Arabs with PD [320]. Finally, mutations in the glucocerebrosidase gene are more often present in PD subjects relative to controls (OR, 5.43), indicating that mutations in these genes are strongly associated with development of PD [321] and can be suggested to the used as a diagnostic markers for PD.
1.4.11.6. Tissue based biomarkers

A simple blood test for PD would be ideal, but despite some promising candidates, there is no blood, CSF, or tissue-based (e.g., skin) measure that has been proven and widely validated as a diagnostic marker in PD. A biomarker does not need to be specific for PD to serve a useful purpose, however, given that some biomarkers may track disease progression (e.g., urinary 8-hydroxydeoxyguanosine [8-OHdG] or serum uric acid) but are nonspecific for PD. α-synuclein being one of the most studied.

1.4.11.7. α-Synuclein

α-Synuclein is the major component of Lewy bodies, it can be readily measured in plasma, and increased levels of α-synuclein oligomers in plasma appear to have good specificity (85%) for detecting PD compared with controls in some studies [322]. α-Synuclein levels appear decreased in CSF in subjects with synucleinopathies [323]. PD patients appear to have increased expression of α-synuclein in their skin fibroblasts, a potentially easily accessible tissue in which this might serve as a diagnostic biomarker [324].

1.4.11.8. Oxidative stress markers

Oxidative stress is increased in PD but is not specific to this condition. 8-OHdG, nitrotyrosine, and reactive oxygen species may be useful biomarkers for tracking disease progression in PD. Ihara et al. [325] found that hydroxyl radical levels also were significantly higher in the plasma of PD patients relative to controls and were correlated with disease duration and Hoehn and Yahr stage. Oxidative stress biomarkers can be assayed easily in blood and urine using enzyme-linked immunosorbent assay (ELISA) or more sensitive methods. However, a variety of conditions alter oxidative stress in a given patient (e.g., normal aging, smoking,
vigorose exercise, antioxidants, drugs, cancer, and chemotherapy), and these may be hard to control.

1.4.12. ASSESSMENT OF PARKINSON DISEASE MANIFESTATIONS

There are several approaches used for clinical assessment of all of these manifestations of PD, and the most widely used are UPDRS. Some scales assess motor manifestations or complications of treatment, and ratings may vary tremendously depending upon whether observations are recorded during the ON or OFF phase for those with such medication-induced fluctuations.

1.4.12.1. RATING SCALE

1.4.12.1.1. Unified Parkinson disease rating scale (UPDRS)

Multiple different scales for PD have been developed for quantification of motor manifestations (Webster, Columbia University Rating Scale, and Parkinson’s disease Impairment Scale); disability (Schwab and England and Northwestern University Disability Scale); or both (UPDRS and New York University Scale) [326]. Of these different scales, the UPDRS has gained the greatest acceptance as a tool for evaluation of interventions and as a clinical tool to follow patients [327]. However, there are important limitations to this scale [328, 329] and a new UPDRS is undergoing validation testing [330]. The current UPDRS includes four subscales. Subscale 1 covers mentation, behavior, and mood. Subscale 2 rates activities of daily living. Subscale 3 is a clinician rating of the motor manifestations of PD. Subscale 4 covers complications of therapy. Data for subscales 1, 2, and 4 are elicited from patients and caregivers, whereas data for subscale 3 is examination-based. There are training tapes for the UPDRS subscales 2 [331] and 3 [332], and reviewing these can improve the reliability of the measures [333]. However, reliability of the other subscales depends on patient reporting in
addition to examiner skills but there is a training tape for the activities of daily living component subscale 2 [331]. The total UPDRS score and the UPDRS subscale scores are not interval scales, which means that there are not quantified, equal distances between values on these scales. For example, a score of 4 is greater than 2 but does not necessarily indicate twice the degree of severity. Each part of the rating is a rank order measure rather than a precise interval change.

**UPDRS Subscale 1: Mentation, Behavior, and Mood**

The examiner asks the patient about each of the following areas of cognitive function or mood and the rater scores the answers from 0 to 4, with 4 representing the greatest level of dysfunction, based upon the responses of the patient or a caregiver. The sum of these scores for this subscale can range from 0 (normal) to 16.

1. Intellectual impairment. Possible ratings of patient response:
   0–none
   1–mild consistent forgetfulness with partial recollection of events and no other problems
   2–moderate memory loss, with disorientation and moderate difficulty handling complex problems
   3–severe memory loss with disorientation with respect to time and often place; severe difficulty with complex problems
   4–severe memory loss with orientation preserved only to person; unable to make judgments or solve problems, cannot be left home alone

2. Thought disorder. Possible ratings of patient response:
   0–none
   1–vivid dreaming
   2–benign hallucinations with insight preserved, that is, the patient is able to distinguish that the hallucinations are not real
3–occasional to frequent hallucinations or delusions with preserved insight; could interfere with activities of daily living
4–persistent hallucinations, delusions or florid psychosis, not able to care for self

3. Depression. Possible ratings of patient response:
   0–not present
   1–periods of sadness or guilt more than normal, but not sustained for more than 1 week
   2–sustained depression greater than 1 week
   3–sustained depression with vegetative symptoms
   4–sustained depression with vegetative symptoms and with suicidal thoughts or intentions

4. Motivation and initiative. Possible ratings of patient response:
   0–normal
   1–less assertive than usual, more passive
   2–loss of initiative or disinterest in elective activities (e.g., less interest in hobbies or social activities, tends to stay at home)
   3–loss of initiative or disinterest in day-to-day activities (e.g., less attentive to personal hygiene, dressing)
   4–withdrawn, complete loss of motivation (e.g., may sit in a chair most of the day with little initiative)

**UPDRS Subscale 2: Activities of Daily Living**

The examiner asks the patient to describe his or her function separately in the ON and OFF state. The responses for each of the 14 items on subscale 2 are therefore scored twice, once for ON and once for OFF. These ratings are done by the examiner based upon the responses of the patient or caregiver. The total score for subscale 2 ranges from 0 to 56.
1. Speech:
   0–normal
   1–mildly affected, with no difficulty being understood
   2–moderately affected, occasionally asked to repeat statements
   3–severely affected and frequently asked to repeat statements
   4–unintelligible most of the time

2. Salivation:
   0–normal
   1–slight but definite excess of saliva; may have nighttime drooling
   2–moderate excessive saliva; may have minimal daytime drooling
   3–marked excessive saliva; some daytime drooling
   4–marked drooling; requires constant use of tissue or handkerchief

3. Swallowing:
   0–normal
   1–rare choking
   2–occasional choking
   3–requires soft food
   4–requires nasogastric tube or gastroscopy tube for feeding

4. Handwriting:
   0–normal
   1–slightly slow or small
   2–moderately slow or small; all words are legible
   3–severely affected; not all words are legible
   4–the majority of the words are not legible

5. Cutting food and handling utensils:
   0–normal
   1–somewhat slow and clumsy but no help needed
   2–can cut most foods; although clumsy and slow; some help needed
   3–foods must be cut by someone, but can still feed self slowly
4—needs to be fed

6. Dressing:
   0—normal
   1—somewhat slow, but no help needed
   2—occasional assistance needed with buttoning and putting arms into sleeves
   3—considerable help required, but can do some things alone
   4—completely dependent upon assistance; unable to help

7. Hygiene:
   0—normal
   1—somewhat slow but no help needed
   2—needs help to shower or bathe; very slow in hygienic care
   3—requires assistance for washing, brushing teeth, combing hair, using the toilet
   4—needs bladder catheter or other mechanical means

8. Turning in bed and adjusting bedclothes:
   0—normal
   1—somewhat slow and clumsy but no help needed
   2—can turn alone or adjust sheets, but with great difficulty
   3—can initiate attempt, but cannot turn or adjust sheets alone
   4—cannot turn in bed or move a small amount to begin a turn or adjust bedclothes

9. Falling (unrelated to freezing):
   0—none
   1—rare falling
   2—occasional falls; less than once daily
   3—falls on average once daily
   4—falls more than once a day

10. Freezing when walking:
    0—none
    1—rare freezing when walking; may have start hesitation
2–occasional freezing when walking
3–frequent freezing; occasional falls due to freezing
4–frequently falls due to freezing

11. Walking:
0–normal
1–mild difficulty; may not swing arms or tends to drag a leg
2–moderate difficulty but requires little or no assistance
3–severe disturbance of walking; requires assistance
4–cannot walk at all, even with assistance

12. Tremor in right arm:
0–absent
1–slight and infrequently present; not bothersome to patient
2–moderate, bothersome to patient
3–severe; interferes with many activities
4–marked; interferes with most activities

13. Tremor in left arm:
0–absent
1–slight and infrequently present; not bothersome to patient
2–moderate, bothersome to patient
3–severe; interferes with many activities
4–marked; interferes with most activities

14. Sensory complaints related to parkinsonism:
0–none
1–occasionally has numbness, tingling, or mild aching
2–frequently has numbness, tingling, or aching; not distressing
3–frequent painful sensations
4–excruciating pain
UPDRS Subscale 3: Motor Examination

Subscale 3 is an examiner rating of the motor manifestations of PD. This is the most commonly used subscale and has 14 different types of ratings, with many of these ratings done independently for the different limbs. Each of the ratings ranges from 0 to 4. The original UPDRS included only integers, but some use 0.5 increments; however, use of these 0.5 increments has not undergone clinicometric testing or validation. The total score for subscale 3 ranges from 0 to 108, the sum of scores from 27 observations.

**Tremor at rest, items 1 to 5**—Tremor at rest is rated in the face and each extremity separately (5 ratings). The typical facial resting tremor appears in the chin, jaw, or lips, and is best observed with the patient seated on a chair. Resting tremor most commonly appears as a flexion-extension movement of the wrist/hand, a pronation-supination movement of the forearm, or a pill-rolling movement of the thumb and index finger. For some people, this tremor may only be apparent while walking. Tremor in the legs may be visible either with the legs at rest while sitting or when lying supine. Ratings are then done for the face and each upper extremity separately using the following scoring:

0–none

1–slight or infrequently present

2–mild in amplitude or persistent, or moderate in amplitude and intermittent

3–moderate in amplitude and present most of the time

4–marked in amplitude and present most of the time

**Action tremor, items 6 and 7**—Action or postural tremor of the hands is assessed with the upper extremities under three conditions. First the upper extremities are fully extended straight ahead at shoulder height with the index fingers of either hand pointing at each other, held closely but not quite touching.
The next position for assessment is with the elbows fully flexed and the arm 
elevated at the patient’s sides (like “chicken wings”) and the index fingers held 
close to either side of the nose, without touching the nose. Finally, the patient 
should be instructed to touch the index finger to the nose and then fully extend the 
limb to use that same index finger to touch the examiner’s finger. This motion 
should be repeated several times with each limb. After all of these maneuvers, a 
single rating is done for each limb for action or postural tremor:

0–none
1–slight, present with action
2–moderate in amplitude, present with action
3–moderate in amplitude, present with posture holding as well as with action
4–marked in amplitude, sufficiently severe to interfere with feeding

Rigidity, items 8 to 12—Rigidity refers to increased resistance to passive 
range of motion and is rated for the neck and all four extremities separately (5 
ratings). Rigidity feels like bending a lead pipe; occasionally there is a ratchetiness 
called cogwheeling that also may be felt. Rigidity is tested in the limbs by 
supporting the joint with one hand; the examiner then uses the other hand to move 
the patient’s limb through a full range of motion. In the upper extremities, this is 
tested at the wrist, elbow, and shoulders with the greatest amount of rigidity 
detected used as the rating for that limb. Rigidity in the lower extremities is usually 
tested by fully extending and flexing the knee with the patient either sitting or lying 
supine. Rigidity at the neck is tested in the seated patient by the examiner placing 
one hand on the forehead and the other hand on the occipital part of the head. The 
head is then gently fully extended and flexed with the degree of resistance assessed. 
If no rigidity is detected at one of these sites, then it should be checked again while 
either having the patient make large circles in the air with a limb on the opposite 
side of the body or while tapping either foot on the floor. These additional
movements increase rigidity. Separate ratings for each of the limbs and the neck are
done using the following scoring:

0–none
1–slight or detectable only when activated by other movements
2–mild to moderate
3–marked but full range of motion easily achieved
4–marked and full range of motion achieved with difficulty

**Bradykinesia, items 13 to 14**—Bradykinesia, or slowness of repetitive
movements, is rated using three different methods in the upper extremities, rating
the left and right separately (and testing each area separately). The first test is finger
tapping. The patient is seated and asked to tap the thumb and index finger, making
the movements as wide and fast as possible. This is done for at least 15 sec with
each hand. The speed of movement and the amplitude are included in a single rating
for each hand.

0–normal (>15/5 sec)
1–mild slowing and/or reduction of amplitude (11 to 14/5 sec)
2–moderate in amplitude; definite early fatiguing; may have occasional arrests of
movement (7 to 10/5 sec)
3–severely impaired; frequent hesitation in initiating movements or arrests in
ongoing movement (3 to 6/5 sec)
4–can barely perform the task (0 to 2/5 sec)

**Bradykinesia, items 15 to 16**—Bradykinesia is also rated with the arms
in the same position as for hand rotation, but this time having the patient open and
close the hand as quickly as possible, also with the largest excursion possible.
Ratings for each hand should be done.

0–normal (>15/5 sec)
1–mild slowing and/or reduction of amplitude (11 to 14/5 sec)
2–moderate in amplitude; definite early fatiguing; may have occasional arrests of movement (7 to 10/5 sec)
3–severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement (3 to 6/5 sec)
4–can barely perform the task (0 to 2/5 sec)

**Bradykinesia, items 17 to 18**—Bradykinesia is also rated for each upper extremity by having the seated patient raise the elbow to the level of the mid-chest, flex it to 90° with the hand pointing up, and then rotate the hand and forearm as rapidly as possibly with the greatest excursion possible. This motion should also continue for 15 sec. This is rated in the same fashion as finger tapping for each side.
0–normal (>15/5 sec)
1–mild slowing and/or reduction of amplitude (11 to 14/5 sec)
2–moderate in amplitude; definite early fatiguing; may have occasional arrests of movement (7 to 10/5 sec)
3–severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement (3 to 6/5 sec)
4–can barely perform the task (0 to 2/5 sec)

**Bradykinesia, items 19 to 20**—Bradykinesia is also tested in the feet with the patient seated and having both feet flat on the floor. Then, each foot is tested separately by having the patient raise the entire foot and lower it back to the floor with as wide excursion as possible and as fast as possible. Some have modified this to have the patient keep the heel on the floor and then repetitively elevate the front of the foot by dorsiflexing the ankle, and lastly return the foot flat on to the floor again using the largest excursion possible and as fast as possible. Each limb is then scored.
0–normal (>15/5 sec)
1–mild slowing and/or reduction of amplitude (11 to 14/5 sec)
2—moderate in amplitude; definite early fatiguing; may have occasional arrests of movement (7 to 10/5 sec)
3—severely impaired; frequent hesitation in initiating movements or arrests in ongoing movement (3 to 6/5 sec)
4—can barely perform the task (0 to 2/5 sec)

**Speech, item 21**—Speech typically becomes soft and indistinct in PD. This is rated by listening to the patient speak, but there are no standard sentences that have been used for this rating. Overall, consistency of this rating is improved by identifying a standard short paragraph for the patient to read and using this same paragraph on subsequent ratings. Speech is scored with a single rating.

0—normal
1—slight loss of expression, diction, or volume
2—monotone, slurred but understandable, moderately impaired
3—marked impairment, difficult to understand
4—unintelligible

**Facial expression, item 22**—Facial expression is rated by observing the spontaneous expressive movements of the face during the entire evaluation. People with PD tend to develop reduced spontaneous facial expression and a mask-like face.

0—normal
1—minimal hypomimia (reduced facial movement); may be a normal “poker face”
2—slight, but definitely abnormal reduction of facial expression
3—moderate hypomimia; lips parted some of the time
4—masked or severe hypomimia with severe or complete loss of facial expression; lips parted ¼ inch or more
**Arising from a chair, item 23**—Ability to arise from a chair is rated by having the patient first cross the arms on the chest and then try to arise from a firm chair that has solid arm rests, but without using the arms to assist, if possible. Degree of disability is rated.

0–normal
1–slow or may need more than one attempt
2–pushes self up from arms of seat
3–may fall back and may have to try more than once, but able to do this independently
4–unable to arise without help

**Posture, item 24**—Posture tends to become increasingly flexed at the neck, shoulders, elbows, wrists, hips, and knees as PD progresses. A single rating for posture as a whole is done.

0–normal
1–not quite erect, slightly stooped; could be normal for an elderly person
2–moderately stooped; definitely abnormal; can be slightly leaning to one side
3–severely stooped with kyphosis; can be moderately leaning to one side
4–marked flexion with extreme abnormality of posture

**Gait, item 25**—As PD progresses, gait is characterized by smaller, slower steps with a shuffling character. Arm swing may be reduced while walking. Some patients tend to shuffle faster and faster while leaning forward as walking continues. This is called propulsion. Others tend to fall and take many steps backwards, so called retropulsion. Festination refers to shuffling, hesitant steps. All of these features are considered in a single rating of gait.

0–normal
1–walks slowly; may shuffle with short steps but no festination or propulsion
2–walks with difficulty but requires little or no assistance; may have some festination, short steps, or propulsion
3–severe disturbance of gait; requires some assistance
4–cannot walk at all, even with assistance

**Postural stability, item 26**—Postural stability is assessed with the pull test. People with PD tend to have increasing difficulty maintaining balance, particularly with a backwards perturbation. The pull test is done to determine an individual’s ability to maintain upright posture with a sudden backwards pull. The test is done as follows. First the patient is asked to stand with the feet shoulder width apart and eyes open. The examiner stands behind the subject in a position that permits the examiner to brace him or herself in case the patient falls backwards during the testing procedure. An examiner can stand with the back close to a wall if there is concern about not being able to catch the patient falling backwards. Once in position, the examiner instructs the patient to resist a backwards pull, but, if needed, step backward to maintain balance. The examiner then gives a small practice pull on the patient’s shoulder. After that, the patient is warned that the next pull will be much harder and then the examiner gives a sudden strong backwards pull on both shoulders. If the patient cannot maintain balance while standing before any pull, then the pull part of the test is not necessary. The pull test is given a single rating.

0–normal (takes two or fewer steps backward to maintain balance)
1–takes more than two backwards but stops self from falling
2–would fall if not caught by the examiner
3–very unstable; tends to lose balance spontaneously
4–unable to stand without assistance

**Body bradykinesia, item 27**—People with PD also tend to have fewer spontaneous movements (so-called akinesia) and generally slow movements. These
are rated for the body as a whole with a single rating (note that akinesia is also rated by facial expression).

0–normal
1–minimal slowness giving movements a deliberate character, possibly reduced amplitude
2–moderate degree of slowness and poverty of movement that is definitely abnormal; alternatively some reduced amplitude
3–moderate slowness, poverty, or small amplitude of movement
4–marked slowness, poverty, or small amplitude of movement

UPDRS Subscale 4: Complications of Therapy

Subscale 4 includes 11 questions that relate to the observations of the patient or caregiver during the preceding 1 week. The first three questions and question 8 are rated from 0 to 4, but the remaining questions are simple no/yes questions. The score on this subscale ranges from 0 to 23. Note, however, that this is a relatively crude measure of dyskinesias and is not based upon direct examiner observation. One important limitation of this approach is that patients may not be aware they are having dyskinesias. In these situations, it will be helpful to interview a caregiver or other observer to obtain collateral information. In fact, it may be necessary to demonstrate those movements to this collateral source.

Dyskinesias

Duration of dyskinesias, item 1: Record the proportion of the waking day during which dyskinesias are present:

0–none
1–1% to 25% of the day
2–26% to 50% of the day
3–51% to 75% of the day
4–76% to 100% of the day

**Disability, item 2:** Rate disability due to dyskinesias:

- 0–not disabling
- 1–mildly disabling
- 2–moderately disabling
- 3–severely disabling
- 4–completely disabling

**Pain, item 3:** Rate pain that the patient associates with dyskinesias:

- 0–not painful
- 1–slightly painful
- 2–moderately painful
- 3–severely painful
- 4–markedly painful

**Early morning dystonia, item 4:** Record presence of early morning dystonia (usually cramping in the legs or twisting of ankle that occurs when first awakening in the morning):

- 0–no
- 1–yes

**Clinical fluctuations**

**Predictability, item 5:** Are any OFF periods predictable as to timing after a dose of medication?

- 0–no
- 1–yes
Unpredictable OFF, item 6: Are any OFF periods unpredictable as to timing after a dose of medication?
   0–no
   1–yes

Sudden OFF, item 7: Do any OFF periods come suddenly (e.g., within a few seconds)?
   0–no
   1–yes

Time OFF, item 8: What proportion of the waking day is the patient OFF, on average?
   0–none
   1–1% to 25% of the day
   2–26% to 50% of the day
   3–51% to 75% of the day
   4–76% to 100% of the day

Other complications

GI complaints, item 9: Does the patient have anorexia, nausea or vomiting?
   0–no
   1–yes

Sleep disturbances, item 10: Does the patient have any sleep disturbances (e.g., insomnia or hypersomnia or (i.e., sleeping through the night or excessive daytime sleepiness)?
   0–no
   1–yes
Orthostasis, item 11: Does the patient have symptomatic orthostasis (i.e., a sense of lightheadedness or actual fainting due to a drop in blood pressure associated with standing)?

0–no
1–yes

1.4.12.1.2. Hoehn and Yahr rating scale

The Hoehn and Yahr staging is probably the most widely known evaluation of people with PD and was first described in 1967. It is really a simple staging from 0 to 5 of the motor manifestations of PD, intended to reflect the degree of progression, and combines features of motor impairment and disability. However, the scale is not linear and may not even be rank order, with some people having greater disability with stage 2 (with substantial bradykinesia but good stability on the pull test) compared to some that have been ranked as stage 3 (that fall on the pull test but have relatively mild bradykinesia and rigidity). The rating was subsequently modified to include two half scores, as noted below, but no clinimetric testing of this modification has been done [328]. Overall, the Hoehn and Yahr staging is best used for a description of subject groups. This rating is based upon examination of the patient.

0–no signs of disease
1–unilateral disease (on one side)
1.5–unilateral disease plus axial involvement
2–bilateral disease, without impairment of balance
2.5–bilateral disease, with recovery on the pull test
3–mild to moderate bilateral disease; needs assistance to prevent falling on pull test; physically independent
4–severe disability, but still able to walk or stand unassisted
5–wheelchair-bound or bedridden unless aided
The major strength of this scale is that it is well known and the tests are easily performed. However, this is a categorical scale and may not always be rank order, i.e., someone with stage 2 who is very slow with much tremor but has intact postural reflexes may be more impaired for activities of daily living than someone rated as stage 3 who is not nearly as slow or tremulous but falls on the pull test.

1.4.12.1.3. Schwab and England ADL scale

The Schwab and England Scale is an “activities of daily living” (ADL) scale frequently used to provide a single estimate of the patient’s ability to function. The rating is done by the examiner interviewing the patient and, frequently, a collateral source, such as a spouse. This rating varies from 0 to 100% using 5% increments. 100%—completely independent; able to do all chores without slowness, difficulty, or impairment; essentially normal; unaware of any difficulty. 90%—completely independent and able to do all chores with some degree of slowness, difficulty, or impairment; some activities might take twice as long; beginning to be aware of difficulty. 80%—completely independent in most chores; some activities take twice as long; conscious of difficulty and slowness. 70%—not completely independent; more difficulty with some chores; some tasks now take three to four times as long; must spend a large part of the day with chores. 60%—some dependency; can do most chores, but exceedingly slowly and with much effort; some tasks cannot be done; common errors. 50%—more dependent; needs help with about half of activities; slower and experiencing difficulty with all tasks. 40%—very dependent, but still able to assist with all chores; however, few can be done independently. 30%—all tasks require much effort; a few chores can be done alone or at least started alone; much assistance needed. 20%—no tasks done independently; patient can provide slight help with some chores; but requires substantial assistance for all activities. 10%—totally dependent and requires assistance with all activities of
daily living. 0%-vegetative functions with loss of control of swallowing, bladder, and bowel functions.

1.4.13. CURRENT TRENDS IN THERAPEUTICS

Alleviation of parkinsonian symptoms and functional disability is the principal goal of PD management in clinical practice. Most patients in early stages of idiopathic PD will improve in response to medications that are directed at correction of the hypo-dopaminergic biochemical deficit and enhancement of dopaminergic neurotransmission. This approach constitutes symptomatic therapy of PD, but the majority of PD patients will gradually deteriorate. It is thought that an ongoing apoptotic death of dopaminergic neurons in SN underpins this relentless natural history of PD.

1.4.13. 1. SYMPTOMATIC THERAPY

1.4.13. 1.1. Levodopa

At present, Levodopa (L-DOPA or 3, 4-dihydroxy-L-phenylalanine) is the most useful drug for symptomatic treatment of idiopathic PD. Unlike DA, L-DOPA crosses the BBB. After oral administration L-DOPA is taken up by the dopaminergic neurons and converted into DA by the enzyme Aromatic Amino Acid Decarboxylase (AADC). L-DOPA effectively alleviates PD symptoms in the early stages of disease. The current “storage hypothesis” holds that at this stage of PD the available dopaminergic neurons and pre-synaptic dopaminergic terminals maintain the capacity to process exogenous L-DOPA and carry out physiological handling of synthesized DA [334]. It has been suggested that the benefits of L-DOPA wear off with disease progression and ongoing death of dopaminergic neurons [335]. This view may be misleading due to the inability to discriminate against the treatment effects and the natural progression of the disease. According to the “storage
hypothesis”, in the absence of dopaminergic neurons L-DOPA is metabolized into DA by neural cells that lack “dopaminergic machinery”. As a result DA release becomes pulsatile rather than continuous and eventually leads to post-synaptic changes and development of motor complications [336].

At present there is some evidence that L-DOPA can be neuroprotective to dopaminergic neurons. The Early versus Late Levodopa study (ELLDOPA) indicates some neuroprotection will diminishes striatal innervation [337]. The DATATOP study also suggested that L-DOPA slowed the rate of disease progression [334]. In contrast, in vitro experiments suggest that L-DOPA accelerates degeneration of residual dopaminergic neurons through enhanced oxidative stress. However, L-DOPA was not toxic to dopaminergic neurons in vivo in experimental rodents. Recent human trials presented unequivocal evidence that L-DOPA treatment did not cause clinical deterioration over a period of 40 weeks compared to the placebo [338]. However, the potential long-term effects of L-DOPA on dopaminergic neurons remain unclear.

1.4.13.1.2. Direct agonists of dopaminergic receptors (or Dopamine Agonists)

The rationale for developing this class of drugs was the delivery of continuous stimulation of dopaminergic receptors, thought necessary to prevent development of motor fluctuations in long-term. This approach was put forward as an alternative to L-DOPA treatment, based on the hypothesis that L-DOPA treatment set pulsatile stimulation of postsynaptic dopaminergic receptors and promoted development of motor fluctuations. Numerous in vitro and in vivo laboratory studies have shown neuroprotective potential of dopaminergic agonists that can be mediated via several mechanisms including free radical scavenging [339,340], and anti-oxidative properties [341,342].
Data from human trials are not conclusive as to neuroprotective properties of DA agonists in PD patients, chiefly because it is very difficult to discriminate between symptomatic and putative neuroprotective effects in the settings of clinical trials and requires a sophisticated approach to the design and analysis of the study. However, current experience with PD patients suggests that the impact of direct DA agonists on the natural course of PD may not be of a clinically meaningful magnitude [343].

1.4.13.1.2. MAO-B inhibitors

There are currently two selective irreversible MAO-B inhibitor drugs approved for clinical use, rasagiline (Azilect) and selegiline (Deprenyl). Two isoforms of MAO have been identified, A and B. In the human brain, MAO-B is the predominant isoform responsible for the breakdown of DA. Selective inhibition of MAO-B results in the elevation of synaptosomal DA concentrations. The primary rationale for MAO-B inhibition in PD is enhancement of striatal DA through inhibition of DA metabolism and the role of MAOB inhibitors in symptomatic treatment of PD has been well established [344].

Interestingly, both selegiline and rasagiline possess potent neuroprotective and anti-apoptotic properties that are not related to MAO-B inhibition. This effect has been demonstrated in vitro using primary cultures of cortical neurons; both drugs enhanced survival of dopaminergic neurons. Neuroprotection has been demonstrated in vivo in rodent models of Parkinson’s disease. It has been proposed that stabilization of mitochondrial membranes, enhancement of intracellular anti-oxidant systems and induction of pro-survival genes underlies this effect. Recently, the interest in neuroprotective properties of MAO-B inhibitors has been sparked by the study showing that Selegiline slows progression of PD symptoms by about 35% over 5 years [344]. This suggests that
there may be neuroprotective effects on the nigrostriatal system. Rasagiline has only recently gained FDA approval, but preliminary results have been promising [345].

1.4.13.2. Cell based therapies

The neuropathological and neurochemical alterations of the dopaminergic nigrostriatal system are responsible for the major symptoms of PD. This constitutes the premise of DA cell-replacement therapy, whereby introducing DA-producing cells into the parkinsonian brain might replenish the diminishing levels of DA and alleviate or cure PD. Over the last 20 years there has been an enormous research effort in this field of neuroscience. Swedish neuroscientists pioneered transplantation experiments in the mid-70’s and early 80’s [346,347]. In early transplantation experiments the grafts of DA-producing cells were placed into the striatum because this approach yielded best survival of the grafted cells with subsequent dopaminergic reinnervation of the basal ganglia [348,349]. In parallel, the demand grew for the sources of DA-producing cells. Traditionally, fetal ventral mesencephalic tissue has been used for grafting because this region of the developing brain contains precursors of dopaminergic cells, which differentiate into functional DA producing cells in vivo. Ethical issues essentially preclude large-scale use of the fetal-derived ventral mesencephalic tissue. An alternative approach has been developed, whereby embryonic stem cells or committed neural precursors can undergo directed in vitro differentiation into DA-producing cells, these are then harvested and used for transplantation [350,351]. Several groups in Europe reported that PD symptoms improved following grafting of the fetal mesencephalic tissue into the putamen or head of caudate area of PD patients [352, 353]. Based on the promising preliminary results, NIH funded the first prospective, double blind, placebo-controlled trial in which 40 PD patients received fetal mesencephalic transplants or placebo operations [354]. The functional improvement of participants was assessed 12 months following grafting using the UPDRS. Fetal mesencephalic
transplants induced statistically significant improvement in a cohort of patients under the age of 60. Long-term follow-up of the participants revealed five patients who underwent transplantation developed dystonia and dyskinesia. Another prospective, 24 month, double-blind, placebo-controlled trial of human fetal nigral transplantation [355] failed to detect significant differences between grafted and placebo groups. The incidence of dyskinesias was high in this study affecting almost half of the patients that had received mesencephalic transplants. The two human trials are commonly designated as “proof of concept” studies and the negative result are perceived as compromising the entire concept of the cell-replacement approach. However, several factors have been identified that confound interpretation of the negative results of human transplantation trials and require further clarification: surgical technique needs improvement; non-dopaminergic cells within fetal tissue transplants also have been implicated in post-surgical complications [356, 357]. The current mainstream of research is directed at producing a reliable and standardized population of DA-producing cells such as neural progenitor cells (NPC) that can be used for further transplantation trials.

1.4.13.3. Neurosurgical therapies

Neurosurgical interventions have developed symptomatic treatments for motor related disorders particularly for advanced PD patients with ensuing dyskinesias. With the increasing knowledge of the neuroanatomical circuitry, surgical treatments such as precision surgical ablation (pallidotomy and thalamotomy) and Deep Brain Stimulation (DBS) are favorable procedures due to the shortcomings of pharmacological therapies. Surgical ablation therapy has been used in many instances until the late 1990’s to reduce severe behavioral symptoms such as bradykinesia, dyskinesia, and rigidity and to some extent resting tremor. Targets for functional neurosurgery include the ventral intermediate nucleus (ViN), STN or the internal Globus Pallidus (GPi). It is though that the reduction of GPi
activity through ablative surgery rebalances the inhibitory effect of the abundance in striatal GABA due to the loss of DA production. In many models [358] and also human parkinsonism, STN and GPi ablation have shown to improve behavioral outcomes associated with the disease. The procedure itself is irreversible, with serious complications that could lead to permanent disability particularly impairment of speech and visual modalities. Unilateral pallidotomies are still preformed today without any knowledge of the long term effects of the surgery. While results of bilateral GPi lesions are indicative of a reduction in dyskinesia [358], there is an association with increased risk of inherent adverse side effects [358]. Patients with unilateral subthalamic lesions showed pronounced improvement particularly after surgery [359]. However, efficacy was limited in that tremors would reoccur in about 20% of cases. A current alternative surgical treatment, high frequency DBS, has replaced stereotaxic lesioning. Chronic high frequency stimulation of the STN (via ViM) in a pilot study in 1987, yielded some promising results by a reduction in extra pyramidal side effects [360]. This new treatment paved the way for a new type of functional motor disorder neurosurgery without subsequent adverse side effects associated with surgical ablation. In PD, the motor deficits are attributed to increased neuronal activity within the STN and the GPi. The surgery involves an insertion of an electrode attached to a neurostimulator. The neurostimulator sends out electrical signals that modulate neuronal circuitry in target areas in the brain to inhibit the impulses that give rise to motor dysfunction. The electrode is placed into a region to address a particular motor symptom accordingly. DBS is proven to be an efficacious treatment in studies that target regions of both the STN and GPi. These studies revealed a reduction in symptoms such as tremor, bradykinesia and rigidity [361, 362].

The stimulation of either the STN or the GPi resulted in significant improvements in UPDRS motor scales. The mechanisms of STN-DBS and GPi-DBS are paradoxical and still remain unknown. It was hypothesized that electrical
stimulation of the STN and GPi would suppress these structural inputs [363] and would therefore act as a counteractive measure of DA replacement therapy. Conversely, Stefani and colleagues [364] questioned this mechanism suggesting that STN-DBS increases GPi firing rate and synchronizes the STN activity. In a microdialysis model of PD, cGMP was used as a measure of glutamate transmission and was found to be increased by 6 fold in GPi dialysate [364]. Concurrent electrical stimulation is a reversible procedure with fewer surgical complications compared to its lesioning counterpart. Follow-up studies of bilateral STN-DBS patients showed improvement in motor symptoms [365] suggesting that DBS is a beneficial long term treatment. The DBS is a useful procedure because it allows the stimulation of an affected region without further destruction of brain tissue [366]. At the same time, long term stimulation of the STN could aid in slowing the disease progression. However, this is yet to be confirmed since Hilker and colleagues [367] established that bilateral STN stimulation did not alter the rate of disease progression.

1.4.14. NEW THERAPEUTIC STRATEGIES

Current therapeutics for PD is neither curative nor preventative as they only temporarily alleviate some of the symptoms of the disease. Drug intervention needs to aim at halting the progression of PD. Current treatments of PD are successfully improving quality of life but unfortunately largely without the ability to control or reduce the rate of disease progression. An integrative model that combines the putative fundamental aspects of nigral degeneration is needed for appropriate therapeutic targeting to potentially prevent further DA nigral loss.

1.4.14.1. Gene therapy

With the current knowledge of molecular characterization of vital genes involved in the neurodegenerative process, several research groups have embarked
on using gene therapy to help protect and also repair neuronal damage. Deliverance of protein products is difficult as the BBB limits the transfer to the intended destinations. Genetic manipulation has many advantageous applications with many vehicles aiding in the delivery of the gene target such as viral (lentivirus, adenovirus and herpes virus) or non viral (polyplexes) that can infect cells without inducing inflammatory responses and has the ability to affect both dividing and non dividing cells [368,369]. Furthermore, the regulatory control of an element that allows the expression of the gene is the primary mechanism for genetic manipulation. Whilst many genes have been uncovered acting as potential ‘players’ in the degenerative cascade, this has allowed the makings of *in vivo* gene therapy promising a new future treatment for PD. The conceptual difficulty in this approach comes from the unknown cause of sporadic PD [370]. Once the cause has been identified, gene therapy may then take greater prominence. While this interventional approach is still a new concept, only a few genes have been trialed in animal models of PD. These gene targets include α-synuclein [371] and Parkin [372].

The use of various neurotrophins in support of the nigral neurons has proved effective in various animal models [374]. The Glial cell line-Derived Neurotrophic Factor (GDNF) has shown the propensity to increase the rate of DA neuronal survival under neurotoxic cell culture conditions [375] and in animal models [374]. Reports of GDNF therapy delivered with an adenovirus have been used in animal models and it was found that GDNF can rescue DA cell loss if administered prior to or shortly after delivery of 6-OHDA [376] or MPTP. Significant improvement in motor behavior is a reflection in significant DA cell recovery of function [377, 378] and correlated with a higher level of DA production in the striatum [377]. These higher levels of DA may occur because of increased TH expression [379]. The results from the various clinical trials have not been so definitive. Lang and colleagues [380], showed that there was no significant clinical benefit in the UPDRS in a phase II trial that investigated the effect of intra-putamen
infusion of GDNF, yet similar studies have shown significant progressive improvements in open-label designed trials [381, 382]. It has been debated that Lang and colleagues did not adequately take into account the placebo effect, the catheter design or the rate of delivery of GDNF [380]. Further, recalculation of the statistical power showed that the power of the study was unable to investigate the effects of GDNF in PD [384]. In light of these difficulties, GDNF properties of promoting cell survival have not been adequately tested in trial for neuroprotection therapy as this would require a longer period of evaluation and sophisticated study design.

Preliminary data using gene therapy to target the STN instead the SN has showed some promise as a therapy in PD. Utilizing a viral expression system, an enzyme (glutamic acid decarboxylase) that synthesizes a neurotransmitter (GABA) was surgically introduced into the STN of patients with PD [384]. The rationale of targeting the STN instead of the SN (which is a primary target seen in many gene therapy trials) was to functionally increase the production of GABA to decrease the aberrant increase in signals to the thalamus [384]. Significant improvement was reported clinically with the functional restoration of circuitry and improvements in motor behavior. Whilst successful in a pilot phase, the question of the placebo effect seen in many surgeries has not been adequately addressed [385]. The clinical presentation of PD symptoms occurs when at least 70% of nigral cells are lost. Goals of therapeutic interventions must therefore address the recovery and prevent the progressive nature of neuronal death in the SN. GDNF partially fulfils these criteria and is therefore an interesting target. However, many factors need to be considered: 1) Regulated controlled delivery of gene products 2) Transfection is notoriously difficult and inefficient in neuronal cultured systems and growing concerns of random integration posing a risk of insertion mutagenesis. 3) Adverse immune reaction and 4) How chronic delivery of these “foreign” genetic products will be restricted to the correct brain region. Gene therapy still has a promising
future and remains in an experimental stage. These factors need to be carefully tested before its emergence as an effective therapy for PD.

1.4.14.2. Anti-oxidant based therapies

Over the last decade, neuroprotective approaches for PD have been tried in an attempt to slow the rate of disease progression. There have been a number of intervention strategies focusing on decreasing oxidative stress. Anti-oxidants can be naturally found in the diet in the form of vitamins (A, C and E), polyphenols, flavonoids and carotenoids. Interestingly, reports of dietary intake (such as high intakes of saturated fats and cholesterol) could possibly influence the susceptibility of developing PD [386, 387]. Dietary anti-oxidants can be found highly in fruits, vegetables, green/black teas and red wine [388], it appears that moderate ingestion of these foods results in a reduction in risk of PD [388]. The properties of anti-oxidants possess is the ability to scavenge for free radicals such as the hydroxyl and the O2 radical. ROS damage can be prevented by selected flavonoids and related phenols (polyphenols) by directly inhibiting both the formation of ROS and the enzymes that produce them. A lot of research to date has focused on the properties of phenols found in tea extracts [389]. These potent anti-oxidants have shown to attenuate the toxic effects of 6-OHDA both in cultured PC12 cells [390] and an animal model of PD. The protection with the use of carotenoids and both vitamin C (ascorbate) and E (β-tocopherol) has been seen in cellular based models of oxidative stress [391]. However these findings are inconsistent with the data produced by epidemiological studies. The use of vitamin supplements has been assessed in large cohort studies and found that there was no association with reduced risks of developing PD [392]. Among individuals who have a high intake of foods that were rich in vitamin E showed significant reduction in the associated risks. Other studies had not found this association [393,394]. Clinical based trials have investigated the use of antioxidants in PD patients. One of the first of its kind
Deprenyl, and β-tocopherol Anti-oxidant Therapy of Parkinsonism (DATATOP) evaluated the use of these agents in a controlled clinical trial setting [395]. The study revealed that β- tocopherol did not benefit in slowing down or reducing the severity of symptoms of PD. This result has been suggested to be a cause of slow absorption and poor penetration into the CNS [395]. Animal models of PD using MPTP evinced conflicting views on the effects of vitamin E [396], vitamin C and carotenoids [397]. Vitamin E deficient mice have an increased susceptibility to MPTP which severely affected the SN [398]. Dietary intake of vitamin E, C and carotenoids in the form of some foods remains consistent over a lifetime, and should be regarded and used in a staple diet from an earlier age. The supplementations of these vitamins are yet to be convincing as a therapy to be used at a clinical level.

A potential new anti-oxidant agent coenzyme Q10 seemingly has some promise as a therapy in mitochondrial disorders and neurodegenerative diseases. Improvement in patients with mitochondrial defects is seen biochemically and clinically with coenzyme Q10 treatment [399, 400]. In neurological diseases that show mitochondrial deficit as a clinical and pathological feature, treatment with coenzyme Q10 could be of benefit. Serving as a potent anti-oxidant, coenzyme Q10 is a lipid soluble molecule which sits in the inner membrane of mitochondria and transfers electrons in the electron transport chain [401]. The anti-oxidative properties involve the ability to scavenge and inhibit the formation of ROS [402]. The neuroprotective effects of coenzyme Q10 is seen in many models of neurotoxicity such as rotenone [403] and MPTP [404]. These studies revealed that in animal lesion models, coenzyme Q10 significantly protected against; loss of TH positive cells in the SN; the depletion of striatal DA and the prevention in the formation of α-synuclein aggregates [405]. Phase II PD clinical trial showed a reduction (44%) in motor deficits measured by UPDRS using a maximal dose of 1200mg (per day) [406]. Storch and colleagues [407] attempted to replicate the
earlier study with a withdrawal phase using participants with middle stage PD in a more rigorous study design. The treatment with coenzyme Q10 in this study showed no significant motor improvements at a dose of 300mg a day [407]. It was concluded that dosage is not sufficient enough to have a symptomatic effect at this stage of the disease. Further trials need to explore the protective effects in PD using the anti-oxidant coenzyme Q10 at a high dosage and for an extended period of time.

1.4.14.3. Therapeutics focusing on metals

There has been substantial research into pharmacological interventions that are involved in the modulation of biometals in neurodegenerative disorders. Iron dysregulation seems to play a vital role in disease pathogenesis in PD patients. These disruptions in the iron homeostatic mechanism observed in PD offer the potential for future therapeutic intervention. Controlling the bioavailability of metals could prevent not only the generation of RS through metallo-redox reactions but also the interaction with other known ‘culprit contenders’ such as α-synuclein.

1.4.14.4. Therapies on metal-associated proteins

Ferritin is a protein that regulates iron storage and can potentially remove any free redox active iron that is present within the cell. Transgenic mice that express high levels of H-Ferritin have been shown to effectively protect the further loss of nigral cells in MPTP [408] and paraquat [409] animal models of PD. H-Ferritin possesses a modifying oxidase activity, which sequesters the iron and converts it to the less bioreactive form [408]. This increase in ferroxidase activity reduces the free iron pool preventing its further participation in redox chemistry [408].
1.4.14.5. Metal chelation therapies

The primary mechanism of chelators is to chemically bind metal ions to form complexes rendering the ions less reactive and allow removal of these ions via the bloodstream for excretion. Pharmacological chelators such as desferroxamine have shown some promise in modulating metal ions. In vitro studies have shown that Desferal intervenes in mitochondrial inhibition by directly enhancing the activation of NADH dehydrogenase [409]. Unfortunately, desferroxamine has poor penetration through the BBB [410]. New iron chelators such as VK-28 were synthesized in order to overcome potential barrier impermeability. VK-28 has been shown to protect nigral cells against 6-OHDA induced lesions [411]. In an induced proteasome dysfunction animal model, chelation therapy with desferroxamine reduced the inhibitory effects on proteasome inhibitors [410]. Iron potentially acerbates the rapid formation of the α-synuclein structure to promote high molecular weight insoluble aggregates. Proteasome dysfunction in this model of PD is relieved by sequestering iron to prevent this aggregation from occurring and protect the nigral cells [410]. Pyridoxal Isonicotinoyl Hyrdazone (PIH) and 2-Pyridylcarboxaldehyde isonicotinoyl Hydrazine (PCIH) share similar potency to desferroxamine, possess high and potent chelating activity, have the ability to cross the BBB and are highly specific for iron overload diseases [412]. PIH and its analogues seem to act in a dose-dependant manner in the immobilization of iron from ferritin and allowing excretion [413].

1.4.14.6. Metal protein attenuating compounds (MPAC)

Metal protein attenuating compounds (MPAC) may offer future therapies for PD. Clioquinol (5-chloro-7-iodo-8- hydroxyquinoline, CQ) is the prototype MPAC and acts by competing with proteins for metal ions [414]. Clioquinol (CQ) is an orally bioavailable drug with moderate affinity for copper, zinc and iron. Differing from traditional chelators as mentioned above, these compounds do not
remove metals from tissues. CQ appears to act as an ionophore to redistribute metals from areas of superabundance to those which may be deficient. Unlike traditional chelators such as EDTA, CQ does not cause bulk excretion of metals but permeates the BBB and potently inhibits metal-mediated hydrogen peroxide production [415]. CQ and analogues are being investigated in a number of conditions in which oxidative stress is a feature. These include, cancer [416] stroke [417], AD [418] and PD [419]. This type of therapeutic approach using CQ type MPACs appears to be encouraging for AD. Animal trials with 21 month old transgenic mice over expressing the amyloid precursor protein (APP) with the Swedish mutation showed a significant reduction in plaques after treatment with CQ [420]. A phase II double-blinded clinical trial showed that CQ treatment for 36 weeks resulted in a reduction of plaque in plasma, with minimal cognitive decline [421]. While this study had a very small subset which reflected within the non significant difference between the groups, these results support the idea that metals play an important role in neurological diseases.

There is also proof of concept that MPACs may be useful for PD therapeutics. A parkinsonian animal model study showed that treatment of animals with CQ for eight weeks prior to induction of lesions resulted in 50% decrease in nigral cell loss compared to animals treated with the parkinsonian toxin MPTP alone [422]. An 8 week pretreatment of CQ resulted in reduction in iron within the SN in MPTP lesioned mice [423]. More recently data showing that CQ treatment commencing only 6 hours after induction of the lesion is equally effective at attenuating SN lesions provoked by intra-nigral injection of 6-OHDA [424] Further the data from a neuronal cell line that expresses the A30P mutant human α-synuclein was rescued by either catalase or CQ [424].
Together with all these studies there are several research are need to be carried out in PD to solve the definite problem start from agent causing PD to treatment of PD. Our research is focused on the early diagnosis of Parkinson’s disease from blood tissue this is an ultimate opening towards human importance to bring out more feasible and rapid diagnosis of PD.