

# Genetics in Parkinson's disease

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Other publications:

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# I INTRODUCTION

## 1 PARKINSON'S DISEASE

### 1.1 Historical perspective and nomenclature

The first clinical description of the disease was published in 1817 by James Parkinson in his “an essay on the shaking palsy” [1]. But it took over 50 years until Jean-Martin Charcot in his teaching at the Salpêtrière Hospital described the four cardinal features of the disease: tremor, rigidity, bradykinesia and postural instability [2].

Pathomorphological changes like selective cell loss, depigmentation and degeneration of the substantia nigra were described in 1953 [3] while the biochemical and pharmacological discoveries like the dopaminergic deficit were discovered in the 1960s [4-6]. Effective neurotransmitter replacement therapy with levodopa was attained in the 1970s. Beside pharmacological therapy surgical interventions have been established as treating potentialities [7-9]. Recently progress in the understanding of the pathogenesis and aetiology of the disease has been achieved highlighting genetic aspects of disease and the protein degradation over the ubiquitin-proteasome system [10]. In therapeutical aspects research is focussing on neuroprotective and neuroregenerative approaches [11].

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## 1.2 Clinical manifestations: diagnosis, motor signs, neuropsychiatric symptoms, other non-motor problems

### 1.2.1 Diagnosis

Parkinson's disease (PD) is characterized through the cardinal features resting tremor, bradykinesia, rigidity and loss of postural reflexes [12]. Discrimination between idiopathic PD and other parkinsonian syndromes is difficult and neuropathological studies have shown that about 20% of the patients diagnosed as idiopathic PD had alternative causes to their parkinsonian syndrome [13-15] reflecting a variety of etiological causes (table 1). Idiopathic PD shows characteristic pathomorphological changes in the brain like cell loss and depigmentation in pigmented brain stem nuclei. Furthermore in most cases eosinophilic, intracytoplasmatic inclusion bodies, termed Lewy bodies are found in the brain. As biological markers of PD do not yet exist and pathomorphological diagnosis is only possible post-mortem clinical features have to be guidelines for exact diagnosis and discrimination of the entities. Beside the cardinal features additional clinical signs help to distinguish PD from other parkinsonian syndromes: asymmetry of parkinsonian signs, marked rest tremor and clinically significant response to levodopa are more likely to be seen in idiopathic PD as well as balance problems in the first years of the disease are uncommon [16, 17]. Clinically idiopathic PD is divided in distinct subgroups concerning clinical unambiguousness [18]. According to clinical manifestations differentiation is done in tremor-dominant versus akinetic/rigid-predominant variants and in young-onset (21-40 years) versus late-onset (>40 years) forms [19].

Accuracy in diagnosis is important and has substantial impact both on prognosis and treatment capabilities.

Table 1: Differential diagnosis in parkinsonian syndromes:

I: Idiopathic (primary) PD

Sporadic PD

Hereditary forms of PD

II: Symptomatic (secondary) PD

Postencephalitic, e.g. encephalitis lethargica, AIDS encephalitis

Vascular

Toxic induced, e.g. manganese, carbon monoxide, MPTP

Drug induced, e.g. neuroleptics, antiemetics

Traumatic

Metabolic, e.g. Wilson's disease

Neoplastic

Normal pressure hydrocephalus

III: Other neurodegenerative diseases

Multi system atrophy (MSA)

Progressive supranuclear palsy (PSP)

Corticobasal degeneration (CBD)

Dementia with Lewy-bodies (DLB)

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Pallidonigral degeneration, e.g. Hallevorden-Spatz disease

Huntington disease (Westphal variant)

Fragile x-syndrome

### 1.2.2 Motor features

The typical parkinsonian **tremor** is a rest-tremor with a frequency of 4-6 Hz. The tremor diminishes during activity and disappears during sleep. Relaxation improves and mental or physical stress deteriorates the symptomatology. Only half of the patients present with tremor and 15% do not develop tremor in the course of their disease. Typically it starts in one extremity and spreads to both ipsi- and contralateral body parts. Beside the tremor PD-patients develop an increasing resistance against passive muscle stretch, the so called **rigidity**. In the clinical investigation the increased muscle tone and the underlying tremor leads to the typical “cogwheel phenomenon”. Rigidity deteriorates during mental stress and might be accreted by active or passive movement of the contralateral limb. Accompanying slowness of movement (and amplitude) is called **bradykinesia** or sometimes synonymously hypokinesia and akinesia. Clinically bradykinesia might manifestate as delay in arresting movement (prepulsion), delay in acceleration of movement (festination), and inability to initiate movement (start hesitation) or sudden transient freezing. As last cardinal sign **postural instability** describes the loss of reflexes causing propulsion or retropulsion leading to frequent falling. Postural abnormality is leading to a flexed posture of body and limbs.

At the beginning of the disease parkinsonian signs may be subtle and comprise slowness and problems with hand-writing. In moderate and advanced state patients develop increasing gait difficulty, bradykinesia, and tremor. In later stage risk of falling with secondarily violation becomes an urgent clinical issue [20]. After five years of treatment motor and non-motor fluctuations, dyskinesias, and behavioural changes become increasingly frequent [21]. Motor fluctuations comprise decline in motor performance near the end of each medication dose (wearing off), fluctuations from good motor to poor motor performance with partially immobilization over seconds (on-off periods, sudden on-off), involuntary movements at peak dose concentration and at the end of the dose (peak-dose and end of dose dyskinesias) [22]. Other motor features explained by the cardinal signs are hypomimia, hypophonia, micrographia, and difficulty turning in bed.

### 1.2.3 Neuropsychiatric symptoms

Behavioural and cognitive changes occur often in the course of the disease. Depression is seen in approximately one third of the patients [23-26] and does not correlate with motor symptoms [27]. Psychotic behaviour and hallucinations occur as well in about one third of the patients and hallucinations prevalently as visual experiences [28]. Dementia is 6 times more abundant in PD patients as compared to the general population [29] and the results from several recent studies strongly indicate that limbic and cortical Lewy bodies are the main cause of dementia in PD [30, 31]. Dementia is a key symptom for PD patients as it increases the risk of nursing home admission [32], mortality [33] and has a substantial impact on quality



of life for patients and caregivers. Non-specific fatigue occurs in about 50% of the patients [34] and is an important issue in treatment [35]. Sleep disorders comprise insomnia, daytime sleepiness and REM sleep behaviour disorder [36-39] and are frequently encountered.

#### 1.2.4 Other non-motor symptoms

Olfactory loss is seen even early in the course of the disease and is related to cell loss in the cortical nuclei of the amygdale complex [40, 41]. Other non-motor features include urological problems such as urgency, nocturia, and sexual dysfunction [42, 43]. Other relevant autonomic signs encompass orthostatic hypotension and progressive cardiac sympathetic denervation [44, 45]. Constipation is caused by slow colonic transit and decreased rectal contractions [46]. Skin problems might develop due to increased sebum excretion and seborrheic dermatitis [47]. Last but not least a variety of pain syndromes have been described [48].

### 1.3 Epidemiology

#### 1.3.1 Prevalence

Besides essential tremor PD is the most common movement disorder with a prevalence of 100-150 patients per 100 000 inhabitants. Prevalence is increasing with increasing age as 1% of the population > 60 years and 3% of the population >80 years is affected by the disease [49-51]. There is a modest male predominance (1.5:1)

whereas the gender prevalence differences are not yet explained [52]. There is no substantial difference in prevalence across European countries [53].

### 1.3.2 Incidence

The incidence rates in different cultures are varying partly related to different diagnostic criteria [54, 55]. Most conducted studies found an annual incidence rate between 10 and 20 per 100 000 inhabitants [56, 57].

### 1.3.3 Epidemiological risk factors

Epidemiological studies elaborated that PD is more common in highly industrialized countries and more frequent in Europe and North America than in the Far East [58-60]. As risk factors for developing PD rural living, pesticide or herbicide exposure, and well water drinking are discussed whereas cigarette smoking, coffee, and alcohol consumption are negatively associated [61-64].

## 1.4 Pathology

### 1.4.1 Neuronal death

Neuropathological changes in PD are on the one hand characterised by preceding depigmentation and progressive death of dopaminergic neurons of the pars compacta of the substantia nigra. Within the pars compacta neuronal loss tends to be greatest in the ventrolateral layer followed by the medial ventral and the dorsal layer [65].

Clinically a parkinsonian syndrome becomes evident when 50% of the dopaminergic neurons in the substantia nigra have died, respectively an abatement of the dopamine concentration in the striatum for about 70-80% [66]. The loss of striatal dopamine is believed to result in the cardinal features bradykinesia and rigidity [67]. Beside the loss of dopaminergic neurons in the substantia nigra also a selected but heterogeneous neuron population is dying including catecholaminergic and serotonergic neurons in the brain-stem nuclei, the cholinergic nucleus basalis of Meynert, hypothalamic neurons, small cortical neurons, neurons of the olfactory bulb, sympathetic ganglia, and parasympathetic ganglia in the intestine.

#### 1.4.2 Lewy bodies

On the other hand Lewy bodies, intracytoplasmic, eosinophilic inclusion bodies are characteristic depositions in surviving neurons [68]. Lewy bodies are typically in brains of PD patients but they are not specific for the diagnosis. Within Lewy bodies there is a dense accumulation of misfolded and aggregated alpha-synuclein, ubiquitin and TorsinA [69-71]. Lewy bodies can also be found diffusely in cerebral cortices in DLB [72] and recently it could be shown that the rate of cognitive decline in PD is significantly correlated with the amount of Lewy body pathology [31]. However cases with clinical typical PD sometimes do not have Lewy body accumulations. Particularly patients with Parkin mutations (*PARK2*) have in most cases an absence of Lewy bodies [73]. Even if substantial progress is made in understanding the molecular structure of Lewy bodies and a cellular model exists that generates inclusion bodies responding to antibody testing as Lewy bodies [74, 75] the

importance of Lewy bodies and their role in the pathogenesis of PD still has to be determined.

## 1.5 Pathogenesis

Although the pathogenesis of PD remains elusive evidence from environmental risk studies and genetic approaches suggest a convergence between energy metabolism and the disposal of damaged proteins in the pathogenesis of PD. The active turnover of damaged proteins is of major importance. Abnormal and misfolded proteins are primarily degraded by the ubiquitin-proteasome-pathway (UPS) [10]. Different conditions can lead to an impaired UPS activity.

### 1.5.1 Mitochondrial dysfunction and oxidative stress

Mitochondria are ubiquitous and pivotal in cellular metabolism. Neurons have a high mitochondrial mass and disruption of mitochondrial complex activity is known to cause PD-like symptoms [76]. Administration of environmental toxins such as N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, a derivate of a synthetic opiate) and rotenone (a pesticide) disrupt mitochondrial complex I activity causing energy failure and cell death. In animal models administration of rotenone and proteasomal inhibitors induce PD like symptoms and a selective loss of dopaminergic neurons with accumulation of Lewy bodies in brain areas typically affected in PD patients [77]. These findings demonstrate that mitochondrial and proteasome dysfunction takes a center stage in the pathogenesis of PD and recent genetic evidence

emphasizes the importance of mitochondrial performance [78, 79] (see genetic findings).

Oxidants in neural metabolism are under normal circumstances in a tight regulation as they can cause severe damage. Several oxidants (e.g. hydrogen peroxide, radicals) arise in neural metabolism and react with other molecules (proteins, lipids, nucleic acids) inducing conformational changes and functional disturbances [80]. Even mitochondrial damage as complex I deficiency in PD could be secondary to oxidative stress in the substantia nigra [81, 82].

### 1.5.2 Exotoxins

The role of the exotoxins MPTP and rotenone are discussed in chapter 1.5.1.

Impaired energy metabolism due to a mitochondrial deficit in PD raises the possibility that exotoxicity may contribute to neuronal degeneration. Intracellular calcium levels are known to predict exotoxic cell death by activation of the glutamatergic N-methyl-D-aspartate (NMDA) receptor [83]. Increased calcium levels are buffered by mitochondria and accumulation of calcium in mitochondria followed by mitochondrial depolarization is a critical feature of exotoxic cell death [84, 85] and associated with free radical production [86] and activation of nitric oxide (NO) synthase. NO radicals release iron from ferritin, impair mitochondrial functions through induction of lipid peroxidation and leads to the production of peroxynitrite [87] which appears to be a critical mediator of cell death.

### 1.5.3 Neurotrophic factors

Neurotrophic factors are responsible for growth and survival of neurons during development, and for maintaining adult neurons. Furthermore, they are capable of regenerating damaged neurons in the brain in animal models [88, 89]. The neurotrophin members include glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT3), and neurotrophin 4/5 (NT4/5). Neurotrophins bind to specific tyrosine protein kinase (trk) receptors inducing receptor dimerization at the cell surface followed by phosphorylation of receptor kinase residues. Hereby intracellular proteins are recruited to be involved in signal transduction. These factors initiate survival, proliferation and differentiation in their target cells [90]. The trophic properties of neurotrophins demonstrate their potential for treatment of neurodegenerative diseases like PD. On the other hand, if supply of these neurotrophins is limited or signalling pathways are dysfunctional, this may cause cell death of dopaminergic neurons and contribute in the pathogenesis. Analysis of brain tissue of a PD patient treated with neurotrophins showed induction of neuronal sprouting in human brain underlining the importance of neurotrophins in the pathogenesis of PD and their exciting possibilities for reversing devastating brain disorders [91].

### 1.5.4 Immune factors

The initial association between central inflammation and pathogenesis of PD was derived from post-mortem studies showing a large population of human leukocyte antigen (HLA)-positive reactive microglia cells in midbrains of PD patients [92].

Further studies reported of significantly elevated levels of a variety of proinflammatory factors including complement proteins [93] and cytokines [94-96].

Furthermore, infectious agents have been suspected to be risk factors in PD. A variety of case reports tend to relate viral infections to the development of acute parkinsonism [97, 98] and experimental exposure to Japanese encephalitis virus can induce degeneration of the substantia nigra in animal models [99]. Besides viruses bacterial infections have been proposed to play a crucial role in the pathogenesis of PD. Infections by helicobacter pylori has been associated with the disease [100-103] and infection with a Norcardia strain resulted in apoptotic death of dopaminergic neurons in the substantia nigra [104] supporting a role of inflammatory and infectious mechanisms in the pathogenesis.

### 1.5.5 Traumatic head injury

A possible role of traumatic head injury has been discussed and is controversial. Epidemiologic studies, case reports, and case control studies point to an association between head injuries and development of PD [105-107]. This correlation is strengthened by analysis of a large group of World War II veterans [108]. In contrast several other studies failed to elaborate a correlation between head injuries and development of PD [109, 110]. The underlying mechanisms in trauma-related damage to the dopaminergic neurons remain elusive. Inflammatory processes might

be involved as it could be shown in animal models that head injury is leading to activation of glial cells and an upregulation of various cytokines [111].

## 1.6 Physiology of the basal ganglia

The basal ganglia are a group of anatomically closely related subcortical nuclei associated with modulation of motor function and non-motor-domains. However, despite intensive research there is no single definitive function that can be assigned to the basal ganglia. The basal ganglia are divided in the striatum (putamen, caudate nucleus (NC) , and nucleus accumbens), the external segment of the globus pallidus (GPe), the internal segment of the globus pallidus (GPi), the subthalamic nucleus (STN) and the substantia nigra [pars compacta (SNc) and pars reticulate (SNr)] [112, 113]. The principle circuit of the basal ganglia are cortico-basalganglionic-thalamo-cortical loops. Over this circuit information is collected from cortex areas and routed through the basal ganglia and returned to cortex areas [114, 115].

The striatum is the primary (but not exclusive) input zone to the basal ganglia. The striatum receives input from the entire cortical mantle with a majority of projections from the motor, sensorimotor and prefrontal cortices. Main inputs to the striatum are excitatory and glutaminergic. From the striatum two pathways can be followed further on: on the one hand inhibitory GABAergic connections from the striatum to the SNr and to GPi. These two nuclei are the main output nuclei of the basal ganglia



and connect to the thalamus, a primary target of the basal ganglia. On the other hand connections from the striatum to the GPe exist. From there information is conducted to the STN and finally to the basal ganglia output nuclei (GPi and SNr). Recent evidence suggests that the individual nuclei are more highly interconnected and it is likely that not all loops of connectivity have been determined [115].

Projection from the striatum to the GPi and SNr appears to predominating D1 dopamine receptors whereas in the GPe D2 receptors are the predominating dopamine receptors. Dopamine has different effects on neurons carrying D1 and D2 dopamine receptors, exciting those with D1 receptors and inhibiting those with D2 receptors.

By molecular cloning five different dopamine receptors could be elaborated [116, 117] grouped in 2 classes of dopamine receptors: the D1-like family composed of D1 and D5 and the D2-like family consisting of D2, D3, and D4. Regionally different expression of the dopamine receptors exists: D1 and D2 receptors are expressed abundantly in all regions of the striatum whereas D3 receptors are expressed mainly in the ventral striatum and D4 and D5 receptors are expressed at much lower levels.

## 1.7 Pathophysiology

Lack of striatal dopamine leads to enhanced activity of cholinergic interneurons and consequently to an increased release of acetylcholine. Acetylcholine acts in the

striatum predominantly over central muscarinic receptors and has complex effects to GABAergic striatal projection neurons [118]. Lack of striatal dopamine leads to increased tonic activity in striatal projection neurons to GPe, STN, GPi and SNr. Dysbalances between inhibiting GABAergic and exciting glutaminergic neurotransmission are causative for this change in activity. Besides tonic activity abnormal activity emerges in STN, GPi, and SNr [119-122] leading to enhanced GABAergic inhibition and consequently to oscillatory activity in thalamic areas.

## 1.8 Treatment of patients with Parkinson's disease

To date none of the established therapies are capable to cure the disease. Several agents with neuroprotective potential are being developed or are under study [123]. Thus symptomatic treatment has to be used while waiting for the results of neuroprotective approaches.

### 1.8.1 Levodopa

Unlike the missing neurotransmitter dopamine, its precursor levodopa can cross the blood brain barrier and is converted to receptor accessible dopamine by dopa decarboxylase. Thus exogenous levodopa can replenish the reduced levels of dopamine in the striatum and repair suppressed nigrostriatal dopaminergic neurotransmission. Efficacy of levodopa can be improved by co-administration of inhibitors of peripheral dopa decarboxylase, monoamine oxidase (MAO) and catechyl-o-methyltransferase (COMT) inhibitors. Major problems related to levodopa

therapy are loss of efficacy over time and development of adverse reactions. Orally given levodopa reaches the entire brain and not only the basal ganglia. Besides the dopamine it generates it is handled differently from the endogenous dopamine formed from tyrosine hydroxylation [124]. In contrast to endogenous dopamine the synthesis and release of dopamine from exogenous levodopa is dissociated from neuronal activity. The generated dopamine is not stored in vesicles but spilt out as soon as it is generated leading to a non physiological receptor stimulation and rapid metabolism. This may explain the common adverse side effects including motor fluctuations, dyskinesias, psychosis, nausea, and hypotension [125]. Despite the potential toxicity of levodopa to dopaminergic and non dopaminergic neurons [126] it is still the most effective treatment for PD.

### 1.8.2 Dopamine agonists

Dopamine agonists provide anti-parkinsonian benefits by directly stimulating dopamine receptors [127] and have been shown to protect against the development of levodopa-related motor complications [128-130]. They are about as effective as levodopa in symptomatic treatment of mild-to-moderate PD. In addition, there is a lower tendency to develop motor fluctuations and dyskinesias with agonist treatment than after initiation of therapy with levodopa [131]. Adverse effects of dopamine agonists are similar to those experienced with levodopa including nausea, postural hypotension, and psychiatric symptoms. Furthermore ergot agents are associated with a small risk of tissue fibrosis not noted with the non-ergot dopamine agonists [132, 133]. Transdermal application of the nonergolinic dopamine D3/D2/D1 receptor

agonist rotigotine is leading to stable plasma levels of the agonist and therewith diminishing adverse side effects of therapy [134, 135].

### 1.8.3 COMT-inhibitors

Levodopa is metabolized by different enzymes, including most importantly dopa decarboxylase and COMT. COMT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to the hydroxyl group of catecholamines and is widely distributed including central nervous system neurons and glia, but not nigrostriatal dopamine neurons. When levodopa is administered with a peripheral dopa decarboxylase inhibitor such as carbidopa or benserazide, COMT metabolism of levodopa predominates and levodopa is metabolized to 3-O-methyldopa. Entacapone is mainly acting in the gut and periphery and results in more stable levels in the plasma and thus makes more levodopa available for transport across the blood-brain barrier. Tolcapone also inhibits COMT centrally but can have liver toxic side effects [136]. When COMT inhibitors are added to levodopa therapy, striatal dopamine concentrations increase and efficacy of medication enhances [137, 138].

### 1.8.4 MAO B-inhibitors

MAO is an enzyme that breaks down monoamines and dopamine. Two isoenzymes exist: MAO-A and MAO-B. Isoenzyme B accounts for about 80% of the total MAO activity in the human brain and is responsible for the degradation of dopamine in the striatum [139, 140]. Since this enzyme breaks down dopamine, inhibiting it prolongs the action of dopamine in the brain, and may improve the symptoms of PD. It has

also a mild antidepressant effect. Selegiline is an irreversible MAO-B inhibitor and offers mild symptomatic benefit. Selegiline was the subject of a major neuroprotective trial in PD, the DATATOP trial: initial analysis of the results appeared to indicate that selegiline slowed disease progression but more detailed study pointed as well towards symptomatic effects. Thus, the results of this trial were inconclusive [141, 142]. Rasagiline is a recently developed selective and potent irreversible MAO-B inhibitor. Rasagiline is structurally related to selegiline, but it is more potent and does not have the amphetamine metabolite that selegiline possesses [143]. Clinical trial results suggest that early initiation of rasagiline may slow the progression of impairment associated with PD. However most of its neuroprotective properties have been shown to be distinct from MAO inhibition: rasagiline has direct antioxidant, anti-apoptotic and anti-excitatory effects which are all dose related [144-147].

### 1.8.5 Anticholinergic agents

Anticholinergic medications block cholinergic nerve impulses that help control the muscles of the arms, legs, and body. Furthermore they restrict the action of acetylcholine that helps regulate muscle movement, sweat gland function, and intestinal function. Physiologically effects of acetylcholine and dopamine need to be carefully balanced for normal motor control. In PD the lack of dopamine leads to a chemical imbalance causing symptoms such as tremor and rigidity. Anticholinergics decrease concentration of acetylcholine in order to achieve a closer balance with dopamine concentration. In elderly people with cognitive impairment anticholinergics

should not be used as they can deteriorate symptoms [148]. Thus anticholinergics may be useful in treatment of a carefully selected group of younger people whose main symptom is tremor.

### 1.8.6 Amantadine

Amantadine is an antiviral medication that is also effective in treating some symptoms of PD. Although the mode of action is still not clear it may cause greater amounts of dopamine to be released in the brain, and it may block receptors for acetylcholine [149, 150]. In early stages of the disease amantadine might be more effective than anticholinergic agents at improving bradykinesia and rigidity but less effective at improving tremor. In later stages it may be used to reduce the levodopa dose. Beneficial effect of amantadine has been documented as well in patients with levodopa-induced dyskinesias [151].

### 1.8.7 Surgical approaches

Brain surgery may be considered when medication fails to control symptoms or causes severe adverse events [152].

**Deep brain stimulation (DBS)** is a recently developed technique for treating PD [153, 154]. DBS surgery involves placing a thin metal electrode into one of several possible brain targets and attaching it to a computerized pulse generator, which is implanted under the skin of the chest. Electrical impulses generated affect movement. The theory behind the constant stimulation is that the loss of dopamine producing neurons in PD leads to an abnormal activity in brain nuclei [119-122] offering the

possibility that constant stimulation of these nuclei with a steady-frequency electrical pulse corrects this excessive and abnormal activity. Thus DBS does not affect brain dopamine levels. DBS surgery does not destroy brain tissue and has fewer risks than destructive surgical methods causing this technique to become the preferred surgical method for treating advanced PD. The stimulation of GPi and STN leads usually to improvement in all the symptoms of advanced Parkinson disease; stimulation in vim nucleus of thalamus can effectively treat all types of tremor.

**Pallidotomy** is an older surgical approach based on the hyperactivity of the globus pallidus in PD causing an inhibition of areas in the brain that control movement. Technically in pallidotomy a tiny part of the globus pallidus is destroyed creating a scar. Consequently this reduces brain activity in this area improving movement symptoms as tremor and rigidity.

**Thalamotomy** is rarely done today. Indication is surgical treatment of severe tremor on one side of the body that does not respond to other treatment. No effect is seen on other features like bradykinesia, speech problems, or walking difficulties [16, 17, 155].

**Neurotransplantation** is considered an experimental treatment for Parkinson's disease and not a realistic option at this time [156, 157]. Theoretically fetal brain tissue implanted to the brain is obligated to produce dopamine. Hereby the use of fetal tissue is controversial and future approaches may include transplantation of nerves from other areas in the affected person's own body or from genetically altered cells.

Other approaches include supply of neurotrophic factors [158] and gene therapy models [159].



## 2 GENETIC FINDINGS IN PARKINSON'S DISEASE

### 2.1 Heritability of PD

During the last decade great progress has been made in understanding the genetic basis and mechanisms of neurological diseases. Particular in the understanding of PD recent discovery of genes associated with rare monogenic forms of the disease has provided substantial and novel insight into the molecular disease mechanisms involved. However in the 1980s preferred opinion favoured environmental toxins accountable for the disease. This opinion was strengthened by occurrence of PD in people exposed to MPTP, the protective effect of smoking, and the difference between the prevalence of PD in rural and urban areas [58, 64, 160, 161]. Early twin studies demonstrated a low rate of concordance in monozygotic and dizygotic twins further emphasizing an assumed lack of genetic susceptibility [162]. First in 1999 in an assessment of genetic inheritance in PD by studying the concordance rates of the World War II Veteran Twin Registry it could be concluded that genetic factors are important when the disease starts at or before the age of 50 years [163]. Further discovery of familial forms of PD and elaboration of the genes involved showed clearly that there is a significant genetic component to the disease [164-166].

Molecular evidence from monogenic forms of PD promoted substantial insight in the understanding of specific molecular pathways in PD because monogenic and sporadic forms of parkinsonism share many overlapping features [167, 168] implying that common pathogenic mechanism may underlie disease development.

## 2.2 Genetic factors in PD

### 2.2.1 *alpha-synuclein (PARK1, PARK4)*

The first gene coding for familial PD was identified studying a large kindred from southern Italy (Contursi kindred) with an autosomal dominant transmission of PD. The gene could be linked to chromosome 4q21-q23 [169] and in 1997 an A53T missense mutation in the *alpha-synuclein gene (SNCA)* was identified as the causative mutation. The mutation consists in the transversion at the nucleotide position 209 from guanine to adenine, leading to the change of alanine to threonine in the mutant protein [170]. Affected individuals had typically Levodopa-responsive PD with the same clinical features as seen in sporadic disease forms. Some affected individuals developed additionally marked dementia, orthostatic hypotension, bladder incontinence and myoclonus [171, 172]. Since then two other point mutations in *SNCA* have been elaborated as causative mutations for autosomal dominant disease transmission: the A30P mutation was found in a German family and involves the substitution of guanine to cytosine at position 88 resulting in the change of alanine to proline [173]. Affected individuals display the typical features of Levodopa-responsive Parkinsonism except of early onset dementia. The E46K mutation was found in a Spanish family [174] and affected carriers usually display cognitive decline at an early stage of the disease and show extensive cortical Lewy-body pathology. Recent genetic evidence is indicating a direct correlation between *SNCA* dosage and disease progression [175, 176] and there is some indication that variability in the promoter region of *SNCA* can predispose to PD [177].

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Alpha-synuclein protein consists of 140 amino acids and is concentrated in synaptic terminals [178]. The physiological function of alpha-synuclein is still unknown. Structurally the amino-terminus contains an amphipathic repeat region which can bind to lipid membranes and associates with presynaptic vesicles [179]. This interaction may play a role in regulating synaptic vesicle size, dopamine storage and neurotransmission. The A30P mutation causes a loss of liposome binding leading to a loss of function [180], the E46K mutation causes an increased liposome binding [181] and all three mutations lead to an increased self-aggregation and formation of Lewy-body-like fibrils [182-185]. One proposed mechanism to how alpha-synuclein exerts its neurotoxic effect is through direct impairment of protein degradation over the ubiquitin-proteasome system (UPS) [10]. Other mechanisms have been described as proteasomal inhibition [77], and inhibition of protein degradation over the lysosome/autophagy pathway [186]. Furthermore, overexpression of alpha-synuclein has been linked to mitochondrial dysfunction [187], apoptosis [188], defective cellular trafficking [189] chaperone mediated autophagy [190], increased sensitivity to oxidative stress [191], and dopamine-mediated toxicity [192].

### 2.2.2 *Parkin (PARK2)*

*Parkin* mutations have been first linked to a rare form of autosomal recessive juvenile-onset form of PD in Japanese families [193, 194]. Affected patients display tremor, bradykinesia, rigidity, and have an excellent initial response to levodopa [195, 196]. However some unusual clinical features as dystonia at onset, hyperreflexia, and early treatment related complications may be present. Furthermore,

neuropathological findings are not consistent with idiopathic PD as a lack of Lewy bodies is found [195, 197]. *Parkin*-mutations are common in families with early-onset of PD and are found in up to 50% of early-onset individuals with a positive family history of PD [70]. In 1998 the gene could be located to chromosome 6q25.2-q27 and a homozygous deletion could be detected [194, 198]. Since then a wide variety of *parkin* mutations have been described including deletions, multiplications and missense mutations [199-201]. Although most described cases report an autosomal recessive transmission some cases exist not compatible to recessive inheritance and genetic evidence exists that haploinsufficiency in the *parkin* gene may be a predisposing factor [202, 203].

Physiologically *parkin* encodes for a protein consisting of 465 amino-acids containing an amino-terminal ubiquitin-like domain, a central linker region, and a carboxy-terminal RING domain comprising two RING finger motifs separated by an in-between RING domain [204]. Consistent with the ring finger motif parkin protein acts as an E3 ubiquitin protein ligase [205] in the UPS. Ubiquitination of proteins leads to proteasomal protein degradation. Consequently parkin mutations should lead to an incorrect ubiquitination and an invalid targeting to the proteasome leading to protein accumulation. Surprisingly, *parkin* knockout animal models do not show clinical or pathological hallmarks of the disease [206] but proteomic analysis has instead revealed dysfunction in the mitochondrial oxidative phosphorylation in the ventral midbrain [207] and a decrease of mitochondrial respiratory capacity leading to an

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increase of oxidative damage [208, 209]. Thus *parkin* may have a neuroprotective effect maintaining mitochondrial integrity. Congruously overexpression of *parkin* leads to resistance to mitochondrial dependent apoptosis [210], protection against dopamine mediated toxicity [211], protection against toxicity induced by proteasomal inhibition [212], and protection against loss of dopaminergic neurons [213].

### 2.2.3 Ubiquitin carboxyl-terminal hydrolase L1 (*UCH-L1*, *PARK5*)

*UCH-L1* is a highly abundant neuron-specific protein involved in the regeneration of monomeric ubiquitin in the UPS [214] functioning as an ubiquitin protein ligase [215] maintaining ubiquitin homeostasis [216]. A heterozygous mutation (I93M) has been found in an affected German sibling pair [217]. Because the transmitting parent was asymptomatic the pathogeny of the mutation is still elusive or an incomplete transmission pattern exists. Additionally a heterozygous M124L variant was described in an unaffected individual [218]. The common polymorphism S18Y was reported as underrepresented in a European cohort [219] and thus may have a potential protective effect caused by a reduced ligase activity and contemporaneously normal hydrolase activity not leading to alpha-synuclein accumulation [215]. The protective effect could be affirmed in a metaanalysis of the literature [220]. However no other mutations have been identified to date increasing doubts in pathogeny and leading to the suggestion of benign polymorphism [221]. Compatibility mutant mice lacking functional *UCH-L1* do not develop a parkinsonian phenotype [222]. Yet a possible pathogenic role of mutations in *UCH-L1* gene might reduce availability of free ubiquitin monomers leading to an impaired UPS and protein accumulation [223].

#### 2.2.4 *PINK1 (PARK6)*

In a large Italian family with familial occurrence of PD linkage to chromosome 1p36-37 could be accomplished performing a homozygosity screen [224]. Subsequent mutations in the *PTEN induced kinase 1 (PINK1)* were identified [79]. Affected individuals display young onset but otherwise typical features of PD. Additional screens of early-onset families revealed various novel mutations. However *PINK1* mutations remain less common than *parkin* mutations [225, 226]. *PINK1* is a 581 amino acid protein containing a mitochondrial targeting motif and a kinase domain homologous to serine/threonine kinases of the calcium/calmodulin family [79]. *PINK1* is considered to be a mitochondrial protein with a role in protecting against oxidative stress and apoptosis in in vitro models [226]. Accordingly the G309D mutation is located in the ADP binding site of *PINK1* and impairs the protective effect by harming kinase activity [227]. However the kinase activity has yet to be demonstrated and mitochondrial substrates and interacting proteins have to be identified.

#### 2.2.5 *DJ-1 (Park7)*

Performance of a homozygosity screen in a family with early-onset PD revealed a linkage to chromosome 1p36 [228, 229]. Mutations in the gene encoding for the protein *DJ-1* were found including deletions, missense mutations, and splice site alterations [78, 230]. Affected individuals have a similar phenotype to those affected with *parkin* mutations including dystonia at onset and initial good response to levodopa. Some individuals might exhibit psychosis. *DJ-1* is a homo-dimeric 189

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amino-acid protein of the DJ-1/ThiJ/PfpI superfamily. The prevalence of *DJ-1* mutations is much lower accounting for 1-2% of individuals with familial young onset PD [231]. DJ-1 is expressed ubiquitously including the brain, where it is localized to both neurons and glia [232, 233]. DJ-1 does not appear in Lewy bodies but colocalizes with tau-positive inclusions in several neurodegenerative diseases suggesting a role in distinct neurodegenerative diseases [234, 235]. Interestingly *DJ-1* is depleted in brains of patients with *parkin*-mutations but enhanced in patients with sporadic PD [236]. The physiological function of DJ-1 is unclear but it may have a role in protecting against mitochondrial damage in response to oxidative stress [237]. Furthermore it may protect against endoplasmic reticulum stress, and proteasomal inhibition [238]. The L166P mutation that was found in an Italian kindred leads to an unfolding of the carboxy-terminal region and a loss of dimerization leading to enhanced degradation by the proteasome [233, 239]. Additionally resolution of the dimerization may exhibit direct instability with a consequently abatement of neuroprotective functions [240]. It could be demonstrated that *parkin* associates with mutant DJ-1 supporting its stability [241]. Concordantly oxidative stress enhances interaction linking both proteins in a common neuroprotective pathway. It is suggested that DJ-1 may act as a component of the UPS acting as a chaperone or protease to refold or promote the degradation of misfolded proteins [204, 241].

### 2.2.6 *LRRK2* (*PARK8*)

A linkage to chromosome 12p11.2-q13.1 in a Japanese family with autosomal dominant PD has been identified [242]. The findings up to date suggest that mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene are the most common genetic cause of late-onset PD. Until now frequent *LRRK2* mutations (G2019S, I2020T, L1114L, Q930R, R793M, R1441C, R1441G, R1441H, S1096C, S1228T, Y1699C) have been published [243-246]. The most common mutation so far is the G2019S mutation accounting for 2-6.6% of the autosomal inherited cases with PD, depending on the population investigated [247-251] and 1-2% of the sporadic PD cases [243, 252, 253]. It has been suggested that penetrance of *LRRK2* mutations may be age dependent [248, 251] explaining the reduced penetrance in some affected families. Furthermore as *LRRK2* mutations can be seen as well in asymptomatic individuals as in control subjects an incomplete penetrance is presumed [203]. The R1441G mutation was reported to cause 8% of PD cases in a Basque cohort [243] and the G2019S mutation was not found in any familial PD cases but only in one out of 337 patients with sporadic PD in the study by Berg et al. [244] indicating different frequencies of specific *LRRK2* mutations in different populations. Affected individuals exhibit a clinical phenotype compatible with idiopathic PD but show a wide variety of neuropathological patterns ranging from pure degeneration without Lewy bodies to degeneration with brainstem Lewy bodies, widespread Lewy bodies fitting to pattern seen in DLB, and neurofibrillary tau-positive tangles [242, 246, 254]. However the prevalence of cognitive dysfunction and dementia among *LRRK2* mutation carriers is surprisingly low although the G2019S mutation is located on



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chromosome 12q12, a genetic locus implicated in late onset Alzheimer's disease [255].

At present it still has to be determined how mutations in the *LRRK2* gene cause PD. The *LRRK2* gene contains 51 exons and encodes a protein consisting of 2527 amino-acids called dardain [243]. The protein comprises various highly conserved domains with probable functional attributes and so far it remains unclear which domains play a relevant role in neurodegeneration.

### 2.2.7 Further loci

*PARK3* is a genetic locus for autosomal dominant PD linked to chromosome 2p13 described in two American families descending from southern Denmark/Northern Germany [256, 257]. Penetrance of the mutation is reduced and gene mutations may be widely distributed in the population. Age at onset is similar to sporadic PD.

*PARK10* and *PARK11* have been defined in large population samples and linked to chromosome 1p32 (*PARK10*) [166] and 2q36-q37 (*PARK11*) [258]. These loci represent susceptibility loci which may be important in the pathogenesis of sporadic PD.

*NURR1* (*NR4A2*) is a developmental gene important in development and maintenance of midbrain dopaminergic neurons. Two mutations have been found so far in exon 1 and linkage could be done to chromosome 2q22-q23 [259-261]. At present linkage

has not been described in a single large family and findings could not be replicated.

Pathogenic relevance has to be determined.

*Synphilin-1 (SNCAIP)* is a substrate of the gene product of *parkin* and has been shown to interact directly with alpha-synuclein [262]. It is found in Lewy bodies along with *parkin* and alpha-synuclein. Linkage could be done to chromosome 5q23.1-q23.3 [263]. A direct role for synphilin-1 is suggested by identification of the R621C mutation in two apparently sporadic PD patients of German origin. Both patients reported no family history of PD but genotyping suggested a common ancestor. Functional studies showed that mutant synphilin can form cytoplasmic inclusions. Furthermore transfected cells carrying the R621C mutation are more susceptible to apoptosis than normal control cells.

Candidate genes identified on the basis of their involvement in the dopamine pathway have been accounted as susceptibility genes. Known genes investigated are *MAO B*, *dopamine D2 receptor*, *CYP2D6*, *CYP1A1*, *N-acetyltransferase 2*, *DAT1*, and *glutathione S-transferase M1* genes [264-266]. Up to now the few studies with significant associations between candidate gene and PD have failed to replicate in other samples. Thus the pathological relevance of those candidate genes has to be further examined.

Mitochondrial dysfunction has been implicated in the pathogenesis for a long time. A majority of mitochondrial DNA is dedicated to the reduced nicotin-amide adenine dinucleotide complex I enzyme thus mitochondrial DNA variation might contribute to PD expression. Ten single-nucleotide polymorphisms defining European mitochondrial DNA haplogroups were genotyped for white PD patients and controls. Haplotype J or K were significantly lower associated with PD than haplotypes H suggesting that variation in complex I proteins may be a risk factor in PD susceptibility [267].

## II AIMS OF THE STUDY

The primary objectives of this study were to examine the role of genetic factors and the frequency of known mutations in a population of patients with PD. Further on we intended to examine the role of a genetic influence on disease progression, especially in development of dementia. To obtain this information we have:

- examined the familial occurrence of PD in an unselected group of patients with PD and in two control groups (paper 1).
- studied the impact of *Parkin (Park2)* mutations in our study cohort and compared it to a German group of PD patients and to two control groups (paper 2).
- studied the impact of *PINK1 (Park6)* mutations in our study cohort and compared it to a German group of PD patients and to two control groups (paper 3).
- analysed the genetic contribution to the development of dementia in PD and investigated the molecular basis for the clinical separation of PD with dementia and DLB in an analysis of the literature (paper 4).
- studied the development of dementia in PD-patients with and without familial occurrence of PD and dementia in their families (paper 5).
- examined the role of fragile x premutations in the study cohort and its impact on the development of dementia in the study cohort and in a German group of PD patients (paper 6).

## III SUBJECTS AND METHODS

### 3 Patients and molecular analyses

#### 3.1 Patients

The study population comprised all subjects with PD living in nine municipalities in the southern part of Rogaland, Western Norway on the prevalence day of January 1<sup>st</sup> 1993. Total ascertainment of patients with PD in this geographical area was attempted through a detailed community study in an area of Rogaland county, Norway with 220 858 inhabitants [50]. Clinical information on all patients with suspected parkinsonism was collected from the only available neurological service in the study area and additionally from the general practitioners, nursing homes, physicians in charge of the nursing homes, district nurses, and home health care workers. Information about members of the Rogaland Parkinson's Disease Society was available. After a screening procedure, 400 patients were invited to participate in the study and examined by a neurologist.

Among the 400 patients with possible PD 245 patients (120 men and 125 women) were diagnosed with PD according to explicit diagnostic criteria as defined in the next paragraph [18] (prevalence: 110.9 per 100 000 inhabitants).

### 3.2 Diagnosis and subtypes of PD

All patients were interviewed and examined in an evaluation program consisting of two consecutive one-hour consultations held within one month. The examination program included a diagnostic evaluation of the parkinsonian syndrome evidenced by the patient, based on clinical information at onset of disease, disease development, and the response to levodopa. The patients were classified in groups of [18]:

#### I: Clinical definite idiopathic PD:

A diagnosis of *Clinical definite idiopathic PD* requires that patient must have resting tremor and at least two of the following signs: (1) akinesia or bradykinesia, (2) rigidity, or (3) postural abnormalities. The disease has unilateral onset and development, and the response to dopamine agonism is good to excellent. No significant changes on computed tomographic or magnetic resonance imaging scans should be present.

#### II: Clinical probable idiopathic PD:

For a diagnosis of *Clinical probable idiopathic PD* patients must fulfill at least 2 of the 4 clinical signs from category I. Resting tremor is not obligatory and a maximum of one of the following atypical clinical features is allowed: (1) dementia and/or clinical relevant autonomic failure at onset of the disease, (2) a symmetrical disease presentation, or (3) only a moderate response to dopamine agonists or (4) another atypical feature of idiopathic PD.

### III Clinical possible idiopathic PD:

For a diagnosis of *Clinical possible idiopathic PD* patients must fulfill two of the four cardinal signs. The patients were allowed to have two of the atypical features. The response to dopamine agonists should at least be moderate. All patients should fulfill conventional diagnostic criteria for PD.

### 3.3 Controls

Two control groups, each consisting of 100 individuals of the same age and sex distribution as the patients with PD were taken as clinical controls for the study population. The first group included patients with diabetes mellitus (DM) recruited randomly from the Diabetes Clinic at the Stavanger University Hospital, Stavanger. This group was chosen as a comparative group with another chronic disease and was not a population-based group of patients. The second group was intended to represent a healthy and well-functioning group of elderly and included individuals going to routine visits at their general practitioners (GP). The GPs selected these persons among elderly individuals that were scheduled for half year screening visits and that were without active disease. These patients should not have cancer, major cardiac disease, or any disorder causing important disability.

For genetic correlation a German cohort of PD patients was recruited in Bochum (Germany), following the standard criteria for PD; it consisted of 95 patients suffering mostly from late-onset PD (median age of onset 55.2 years). A positive PD family history was documented in 16.5 % of the patients. Ethnically matched control

samples from healthy blood donors were recruited at the Haukeland University Hospital, Bergen (Norway) and the University Hospital of Essen (Germany).

### 3.4 Clinical evaluation

Information on clinical and demographic patient characteristics was obtained through a semi-structured interview and with rating scales. To obtain strongest possible *certainty* a caregiver with intimate knowledge of the patient and the patient's family accompanied the patient during the interview. Severity of parkinsonism was examined by the Unified Parkinson's Disease Rating Scale (UPDRS) [268], including the Hoehn and Yahr staging [49] and the Schwab and England scale [269].

Hallucinations were rated with the Thought disorder item of the Mental subscale of the UPDRS. A score of 2 and above was defined as visual hallucinations. Dementia was diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) [270] criteria as a guide and taking into consideration the physical disability that occurs in patients with PD. Cognitive functions were rated using the Mini-Mental State Examination (MMSE) [271], symptoms of depression with the Montgomery and Aasberg Depression Rating Scale (MADRS) [272].

#### 3.4.1 Assessment of familial aggregation and heredity of PD

Patients and controls were asked to complete a questionnaire that asked for detailed information about the occurrence of PD and dementia in their families. The relatives



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that should be considered by the patient and/or their caregiver were all siblings, parents, children, siblings of parents, and grandparents.

### 3.5 Molecular analyses

#### 3.5.1 *Park2* and *Pink1*

The exons of the investigated genes were amplified by polymerase chain reaction (PCR) using newly designed as well as established primer pairs. For each PCR reaction, a 10  $\mu$ l reaction mix was set up containing 100 ng DNA, 1x GC buffer (Genecraft, Münster, Germany), 1 U Taq Polymerase (Genecraft, Münster, Germany), 0.2 mmol of each dNTP, 0.4 mmol of each primer and varying concentrations of MgCl<sub>2</sub> (1-3 mmol; Genecraft, Münster, Germany). For SSCP analysis, 0.06  $\mu$ l of [<sup>32</sup>P] dCTP/dATP (10mCi/ml) was added. A touch-down procedure in a thermocycler (Biometra, Goettingen, Germany) was applied: initial denaturation (3 min at 95°C), two initial cycles 6°C and 3°C above the annealing temperature (50°C-55°C) 25-28 cycles of 95°C (30 s), annealing temperature (30 s), elongation at 72°C (30 s) and a final elongation step at 72°C (3 min). In order to optimize mutation screening by SSCP analyses, PCR products were digested with different restriction enzymes depending on the lengths of their fragments. Thereafter, SSCP analysis was used to identify mutations and SNPs. For SSCP analysis, 3  $\mu$ l of PCR product were mixed with 7  $\mu$ l of loading puffer (95% deionised formamide, 10 mM NaOH, 20 mM EDTA, 0.06% xylene cyanol and 0.06% bromophenol blue) and heated for 5 min to 95°C before cooling on ice. 3  $\mu$ l of each mix was run on two sets

of 6% polyacrylamide gels (one set containing 10% glycerol, another containing 5% glycerol and 1M urea) with 1xTBE buffer at 55W for 3-5h at 4°C. Gels were dried and subjected to autoradiography over night. Selected samples with band shifts evidenced in SSCP analyses were confirmed by direct sequencing. The sequence reactions were run on an automated DNA sequencer (Applied Biosystems 377 XL, Foster City, USA) using the Big Dye Terminator kit (BDT; Perkin-Elmer, Norwalk, CT) and analyzed with the ABI Prism™377XL collection and convenient sequencing analysis software. SNP and mutation frequencies in controls were determined by using specific restriction fragment length polymorphism analysis (RFLP) and SSCP analyses.

### ***3.5.2 Fragile x***

The size of the individual CGG repeats was determined by polymerase chain reaction (PCR) as follows: For each PCR reaction, a 10 µl reaction mix was set up containing 100 ng DNA, 1x GC buffer (Genecraft, Münster, Germany), 1 U Taq Polymerase (Genecraft, Münster, Germany), 0.2 mmol of each dNTP, 0.4 mmol of each primer (forward primer 5'-Cy5-CGCTCAGCTCCGTTTCGGTTTCACTTCC GGT-3'; reverse primer 5'-TCCTCCATCTTCTCTTCAGCCCT-3') and varying concentrations of MgCl<sub>2</sub> (1-3 mmol; Qiagen, Germany). PCR was performed with an initial denaturation at 95°C for 15 min, 35 cycles of 95°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 5 min. Repeat length was analyzed on a DNA sequencer using a 400 DNA standard (Beckmann Coulter Ceq 8000).

## IV RESULTS

Paper 1. The frequencies of familial occurrence of PD and dementia were compared: among the 245 patients with PD 30 (12.2%) reported of another first-degree relative with PD compared to 5% and 3% in the diabetes mellitus and the healthy elderly control groups ( $p < 0.001$ ). This difference became even stronger if taken all affected relatives into account: 53 (21.6%) of the patients with PD reported about occurrence of PD in all family compared to 7% and 5% in the control groups. The frequency of dementia in the families of patients with PD, diabetes mellitus and the healthy elderly did not differ neither in first degree relatives nor in all affected relatives. Clinical and demographic factors at baseline were the same in patients with and without PD in their families.

Paper 2. The 12 coding exons of the Parkin gene were amplified: three previously described missense mutations (Thr240Met, Arg402Cys and Arg256Cys) were observed. In the German cohort three different heterozygous mutations (Thr240Met, Arg256Cys and Arg402Cys) were identified, corresponding to a rate of 3.2% mutation carriers, higher than previously described in Caucasian late-onset patients (2%). One healthy control subject carried the Arg402Cys mutation in heterozygous state (44 years at time of testing). In the Norwegian patient cohort one missense mutation was observed in heterozygous state (Arg256Cys), corresponding to a rate of 0.5% mutation carriers. No mutations were detected in the Norwegian control

subjects. Several intronic SNPs (IVS2 + 25T<C, IVS2 + 35G>A, IVS3-20T>C, IVS7-35G>A and IVS8 + 48C>T) were found in both cohorts of patients. The frequencies of the SNPs differed significantly: the SNP IVS2 + 25T>C ( $p=0.015$ ), IVS7-35G>A ( $p=0.013$ ) and IVS8 + 48C>T ( $p=0.029$ ) were more frequent in the German cohorts. Likewise the amino acid changes described previously as polymorphisms (Val380Leu, Ser167Asn and Asp394Asn) were more frequent in the German cohort, except for the Ala82Glu that was identified only in a Norwegian sample. The Val380 allele was significantly more frequent ( $p=0.0081$ ) in the Norwegian cohort. The frequency of Val380Leu polymorphism did not differ between patients and controls.

Paper 3. The 8 coding exons of the *PINK1* gene were screened: Several SNPs were identified: (L63L, Q115L, Ivs4-5A>G het, Ivs6+43C>T het, N521T, c.1783A>T). Allelic frequencies of several SNPs differed significantly between the German and Norwegian cohorts confirming homogeneity of the Norwegian cohort. The screening did not reveal any disease relevant mutation in the two cohorts.

Paper 4. In this analysis of the literature we identified occurrence of coincidental parkinsonism and dementia in 24 families. In 12 of the families it was reported both patients with DLB and with Parkinson's disease dementia (PDD), suggesting a common underlying pathophysiology of the entities. Consequently the distinction

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between DLB and PDD upon a strict separation of time of onset of parkinsonism and dementia does not reflect the molecular biology of the disease process. Patients meeting diagnostic criteria for DLB so far either display mutations in the synuclein gene or show positive correlations with the APOe3/4 and e4/4 allele.

Paper 5. Familial occurrence of PD in first and second degree relatives was associated with occurrence of dementia in PD. 28 (12.8%) PD patients reported of a first degree relative and 23 (10.5%) of a second degree relative affected with PD. 21 (9.6%) reported a first-degree and 21 (9.6%) a second degree relative affected with dementia. The baseline characteristics of these groups did not differ. Dementia was present in 49 patients (22.4%) at baseline, and was diagnosed in another 72 (32.9%) (incident dementia) during follow-up, with a total number of 121 (55.3%).

There was a linear relationship between dementia prevalence and strength of family-association of PD: PD in first-degree relative (75%), second degree (57%), and no family history (52%) ( $p=0.036$ ). However, the Cox hazard analyses failed to detect a significant association between family history of PD and time to develop dementia in PD ( $p>0.05$ ). No association between dementia and family history of dementia could be found although there was a numerical trend towards a higher proportion with dementia in those with dementia in a first-degree (67%) or second-degree (57%) relative compared to no family history of dementia (53%) ( $p=0.64$ ).

Paper 6. No premutation alleles of the fragile X mental retardation (*FMRI*) gene were found. In the Norwegian cohort 2 PD patients (3.6%) and 2 controls (1.2%) had 41-54 CGG repeats ( $p=0.63$ ). In the German cohort 5 PD patients (6.2%) and 3 controls (2.2%) had 41-54 CGG repeats ( $p=0.125$ ). There were no clinical differences at baseline between the patients with more or less than 41 CGG repeats. However the two patients with 41 or more CGG repeats declined 2.33 and 3.22 points in MMSE per year, compared to a mean (SD) decline of only 1.4 (1.3) in patients with less than 41 repeats. Both patients with 41 or more CGG repeats developed severe dementia and hallucinations during the course of the disease.

## V DISCUSSION

### 5 General summary

In our study we clearly show that there is an aggregation of PD in the families of PD patients and we have analysed the frequency of several known mutations. Further on we have investigated whether development of dementia in PD might have genetic determinants. These issues are discussed in detail in the different papers in this thesis. However some methodological concerns and general aspects are of salient importance and will therefore be discussed in the following part of the thesis.

#### 5.1 Methodology

One major methodological issue in research combining clinical and molecular approaches is valid clinical information and reliable molecular elaboration. It is of outstanding importance to have a high diagnostic accuracy of the clinical data. We tried to obtain this by using well defined and published diagnostic criteria [18] during the inclusion period and by using standardized rating scales in the follow-up investigations. Previous studies have shown that 20-30% of patients that were clinically diagnosed as PD had a different cause for their parkinsonian symptoms [13, 14]. To obtain a high diagnostic accuracy the applied classification with subgroups of clinically definite, probable, and possible PD included patients with a high to medium high probability for the disease, whereas patients with a lower probability were excluded. The classification takes both specificity and sensitivity into consideration.

The high diagnostic accuracy in our cohort could be confirmed neuropathologically in a subgroup of the patients that has come to autopsy: all 22 patients had neuronal loss and alpha-synuclein positive Lewy bodies in the surviving neurons of the substantia nigra confirming the diagnosis [31].

A given problem in assessing family history data in neurodegenerative diseases is that accuracy of the obtained data often remains unclear. It was reported that patients with PD tend to overstate PD in their relatives when compared to controls [273]. However a recent study showed that a family history interview can be taken as reliable information when a conservative diagnostic algorithm is used [274]. In order to prevent misclassification and bias-effects in our study cohort we used a semi-structured interview in attendance of a caregiver with intimate knowledge of the patient and the patient's family. Bias effects of the way family history data was collected by can yet not completely be excluded. However, misclassification may occur because relatives are still at risk or died before expression of disease symptoms. Consequently this type of bias leads more likely to an underestimation of associations.

For the study of correlations between clinical progression data and molecular results a prospectively design over a 12-year period in a population based cohort from a cross-sectional study are undisputable strength of the study. However, this may also implicate a major pitfall in interpretation of the devised results. Obtaining clinical data started in 1993 with 245 included patients; four years later only 143 patients could join the follow-up visit, further decreasing to 90 in 2001 and leaving even



fewer patients for the twelve year follow-up. Furthermore, with only 125 blood samples drawn in 2001 the clinical correlations must be even more carefully interpreted. A screening after known mutations is not affected by this prerequisite as no clinical correlation has to be done. In contrast, elaborating new clinical correlations with the gathered progression data remain difficult, caused by the low number of patient data at disposal. However, this methodological problem is as well more likely to underestimate given associations assuring high reliability of obtained correlations.

## 5.2 The role of genetics in the pathogenesis of PD

Still in 1999 PD was largely thought as a sporadic disease caused by age-related and environmental factors with insignificant genetic input [163]. Ensuing epidemiological surveys elaborated a genetic predisposition to sporadic PD [165, 275]. Confirming these results we found a three- to fourfold increased frequency of PD in relatives of PD-patients as compared to controls. As we found an equally increased familial aggregation in both first-degree relatives and in more distant family members a shared environmental exposure represents an implausible explanation for the increased risk. Likewise a higher motivation in PD families for answering questions in the study and an increased interest in the symptoms of the disease are less likely as the control groups showed a good consistency and dementia, as a neurodegenerative symptom, was as common in control families as in PD families.

Further genetic evidence in the pathogenesis of PD was obtained by identification of genes underlying familial occurrence of PD [167]. As up to now genetic PD cases are likely to explain no more than 5-10% of the overall PD population, the relationship between pathogenic principles of familial PD and the common sporadic form remains a key problem in interpretation of devised results. However both familial and sporadic forms of the disease overlap in their clinical syndrome and their characteristic pathology. Hence the understanding of the molecular basis of genetically caused PD may contribute significantly in understanding the molecular biology of both familial and sporadic PD cases with a possible therapeutic potential for the patients.

### 5.2.1 *Parkin*-mutations (*Park2*)

In 1998 mutations in the *parkin* gene were discovered in autosomal-recessive early-onset PD [194]. *Parkin*-mutations are frequent in patients with early-onset PD and it remains controversial whether *parkin*-mutations in a heterozygous state are pathogenic. Several recent studies elaborate that probands carrying single defective *parkin* alleles display reduced 18F-DOPA uptake upon positron emission scanning suggesting that heterozygous *parkin*-mutations may cause sub-clinical nigrostriatal dysfunction [276, 277]. In our study populations we identified four heterozygous mutations, one in the Norwegian cohort (0.5%) and three in the German cohort (1.6%). As our cohorts reflect populations of late-onset PD a low frequency of *parkin*-mutations was expected. However we found a difference in frequency between the study groups. In the Norwegian cohort the number of mutations is a third

of the frequency in the German cohort - reflecting maybe the higher age of onset in the Norwegian cohort (63.6 years vs 55.2 years). The differences of mutations in the screened cohorts may also be caused by different relevance of parkin-mutations in the Norwegian and the German cohorts.

Our data are consistent with previously published studies of affected patients with only one affected allele [203, 278]. Haploinsufficiency is proposed to manifest clinical symptoms by oxidative stress [279, 280] and this model complies with the hypothesis of synergistic influence of genetic and environmental factors in the pathogenesis of PD.

### 5.2.2 *PINK1* (*Park6*)

*PINK1* encodes a mitochondrial protein kinase that appears to have protective effects against oxidative stress. Mutations in the *PINK1* gene are the second most common cause of autosomal-recessively inherited PD after mutations in the *parkin* gene and display an important cause in sporadic early-onset PD [79, 281]. A direct involvement of mitochondrial dysfunction as suggested by discovering pathogenic mutations in *PINK1* is further supported by the fact that MPTP and rotenone can cause Parkinsonism by complex 1 inhibition of the respiratory chain. However a relationship between *PINK1* mutations and late-onset PD as shown for *parkin* mutations could not be demonstrated [282]. In support to these data we could not detect any pathogenic mutation in the screened cohorts representing late-onset

cohorts. However as shown in the *parkin* screen, distribution of genetic frequencies deviated in the two populations as allelic frequencies of several SNPs differed significantly showing homogeneity of the Norwegian cohort. A recently described variation (Q115L) of the *PINK1* gene [283] could be identified in both cohorts and control groups, but failed to show association with PD, confirming previously published data [282].

### 5.3 The role of genetics in development of dementia in PD

PD has traditionally been assumed to be mainly a motor disorder. However the importance of dementia in PD has recently been increasingly recognized and it could be shown that the cumulative frequency of dementia in PD can be as high as 78% [284]. The increasing frequency of dementia in PD may be due to a better understanding and examination of dementia in PD.

Over the last decade important advance has been achieved in understanding the molecular basis of dementia and the relevance of this issue is emphasized by the observation that the increased mortality risk in PD is ascribed largely to the increased risk of becoming demented [285]. Several genes have been identified such as presenilin 1 and 2, Apolipoprotein e (APO e) and amyloid precursor protein involved in the development of Alzheimer's disease. In parallel, the progress in the genetics of PD has enhanced our understanding of basic disease mechanisms highlighting the role of alpha-synuclein: mutations in the *alpha-synuclein* gene giving rise to autosomal dominant transmitted PD [170] and recently a direct relationship between gene dosage of *alpha-synuclein* and disease progression in PD could be shown [176].

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### 5.3.1 *Alpha-synuclein*

Two main entities describing dementia with parkinsonism are differentiated: according to current diagnostic criteria PDD is diagnosed if cognitive decline appears more than one year subsequent to motor symptoms of PD and DLB is diagnosed if dementia precedes motor symptoms or occurs within one year after onset of parkinsonism [286]. DLB is seen as a defined clinical entity characterized clinically by dementia accompanied by parkinsonism, visual hallucinations and fluctuating cognitive impairment.

We analysed the available studies of familial occurrence and genetics of dementia in parkinsonism to elaborate genetic evidence in PDD and DLB, as well as to determine whether there is genetic overlap between the entities. We identified coincidental familial occurrence of dementia and parkinsonism in 24 families. In 12 families presentations of PDD and DLB co-occurred in the same family suggesting an overlap and a shared underlying patho-physiology of the clinical entities. This overlap implies that the present distinction between PDD and DLB, based on the time sequence of onset of parkinsonism or dementia, does not necessarily reflect the molecular biology of the disease process. Interestingly, patients with familial co-occurrence of dementia and parkinsonism displayed either mutations in the *synuclein* gene or showed positive correlations with the *APOε3/4* and *ε4/4* alleles. A direct relationship between occurrence of Lewy bodies and extent of dementia in PD has been shown [31] strengthening the hypothesis that alpha-synuclein accumulation is also a key factor in development of dementia in PD as alpha-synuclein is a substantial

component of the Lewy bodies. Applying the findings that *SNCA* dosage is directly correlated to disease progression [175, 176], this might strongly indicate that degree of genetic alteration is determining clinical severity, i.e. development of dementia. However despite the pathogenic relevance of mutations in *SNCA*, these remain rare causes for familial forms in PD [287].

### 5.3.2 Apolipoprotein e

Another protein that is proposed to influence development of dementia in PD is Apolipoprotein e (Apo e). Apo e is a polymorphic protein involved in lipid transport, immunoregulation, and modulation of cell growth [288]. It is abundant in the brain and coded by the *Apo e* gene located on chromosome 19q13.2. The gene is polymorphic, with three major alleles:  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ , yielding six possible genotypes and translating into three major isoforms of the protein: ApoE2, ApoE3, and ApoE4. These isoforms differ from each other only by single amino-acid substitutions at position 112 and 158 of the protein, but have far reaching physiological implications. The *Apo e*  $\epsilon 3$  is the most common allele. About 95% in the normal Caucasian population carry at least one  $\epsilon 3$  allele [289]. The  $\epsilon 2$  allele is associated with hyperlipoproteinemia [290] and is considered protective in AD [288]. Congruously it has been demonstrated to facilitate neurite outgrowth [288] and to inhibit apoptosis [291]. The  $\epsilon 4$  allele is associated with an increased risk of developing AD and a lower age at disease onset [292-294].

Evidence for the role of *Apo e* in PD has been inconclusive. Contradicting studies have shown associations of PD with the  $\epsilon 2$  allele [295, 296] and of the  $\epsilon 4$  allele with PD [297], PDD [285, 298, 299] and hallucinations or psychosis in PD [300, 301]. Other studies failed to show significant associations [302, 303]. Furthermore an inverse association between the  $\epsilon 3$  allele and PDD has been observed [299].

Interestingly analyses including larger sample sizes elaborated a significant association of the  $\epsilon 4$  allele with early age of onset in PD [304-306]. However other studies failed to show this association [298, 307] and thus competing effects as sample size limitations, differing ethnicities, and publication bias (unpublished negative studies) must be taken into consideration. Yet as larger studies and metaanalyses can show a positive association between *Apo  $\epsilon 4$*  allele and dementia in PD, influence of the  $\epsilon 4$  allele has a high probability. However the mechanisms by which the *Apo e* allele may influence the development of Lewy bodies and dementia in AD and PD remain elusive. Pathologically both diseases comprise the accumulation of insoluble protein deposits and it is suggested that pathologic cascades that lead to protein accumulation may in some cases operate synergistically [308]. As there exists further significant associations of *Apo e* in other neurodegenerative diseases as amyotrophic lateral sclerosis [309] or macular degeneration [310], occurrence of common principles in different neurodegenerative disorders is further strengthened.

### 5.3.3 *LRRK2*

Detection of mutations in *LRRK2* [243, 252] has complicated interpretation further as affected individuals display clinical findings typical for sporadic PD without major development of dementia [244]; the pathomorphologic picture is however remarkably varying, ranging from pure degeneration without Lewy bodies to degeneration with brainstem Lewy bodies, widespread Lewy bodies fitting to the pattern seen in DLB, and neurofibrillary tau-positive tangles [246, 254]. Consequently other influences like neurochemical effects, as the cholinergic deficit, might have an effect on the pathogenesis of dementia in PD as well.

### 5.3.4 Family history

In our study we could demonstrate that a positive family history of PD may emerge as a risk factor for developing dementia in PD. This strengthens the hypothesis that genetic factors contribute in disease progression. Interestingly there was a linear relationship between occurrence of dementia and strength of family-association of PD (first-degree>second degree>no family history) appropriate to a genetic dose dependent effect. Yet we could not find a significant correlation to time of onset of dementia in the the cox-hazard model. This may be influenced by the fact that 49% of our patients had dementia at baseline; thus in this subgroup, the time from onset of PD to dementia was made retrospectively, which is subject to recall bias.

Additionally, since the interval between assessments during the first 8 years of this study was 4 years, the accurate timing of onset of dementia cannot be accurate. These issues may have contributed to the lack of difference in time to develop dementia in



PD patients with or without a family history of PD. Despite the lack of significance, a genetic influence in development of dementia in PD is possible and further studies have to clarify this issue.

A recent study showed a direct correlation between Lewy-body pathology in brain tissues of PD-patients and development of dementia [284]. As accumulation of Lewy bodies shows genetic determinants [182, 311] such factors may also influence the development of dementia in PD. These findings further strengthen a probable correlation between a positive family history of PD and the risk of developing dementia in PD

### 5.3.5 *Fragile X*

Another genetic locus linked to parkinsonism and dementia is the fragile x mental retardation gene (*FMRI*). Premutation carriers of the *FMRI* gene (55-200 CGG repeats) display parkinsonism, cognitive decline and behavioural changes. These issues are also occurring in PD and may therefore represent a potential influence from *FMRI* alleles. Evidence that repeat numbers in the intermediate-size range (41-55 CGG repeats) or in the high normal range (35-40 CGG repeats) [312] may as well play a pathogenic role are strengthening a possible association between CGG repeats of the *FMRI* gene and development of dementia in PD.

We did not detect any premutation carriers showing diagnostic accuracy in our study cohort since carriers of CGG repeats in the premutation range exhibit a distinct

clinical picture preceding mere parkinsonism [313, 314]. Interestingly, both patients carrying intermediate-size alleles developed marked dementia and hallucinations, suggesting a possible association between CGG repeats in the intermediate-size range and cognitive decline in PD. This observation is in line with a report suggesting a gradient pathogenic risk from an allele size for alleles beyond the normal range of ~30 CGG-repeats [312]. As pathogenic model a gain-of-function model is suggested proposing that the degree of degeneration is related to the relative molar quantity according to the repeat length [315, 316]. Yet the number of intermediate-size carriers in our cohort is too low to find statistically significant associations. However the individual cases in our cohort point to a possible association and support the necessity of future studies with larger patient samples.

## VI CONCLUSIONS AND DIRECTIONS FOR FUTURE RESEARCH

It is not certain whether identical disease mechanisms are underlying genetic-caused and sporadic PD, but applying a genetic approach led to identification of common molecular mechanisms in PD. The evidence from genetic studies of PD point to an abnormal protein accumulation and led to realization of the importance of the ubiquitin-proteasome system, the involvement of mitochondrial dysfunction, and oxidatiative stress. In the near future it can be expected that additional PD causative genes will be identified leading to further insight in the molecular level involved. The emerging challenge will be the transfer of molecular insight into clinical practice. Our research results are based on an unselected, community based population with PD that was followed prospectively over a twelve year period and a German PD control population as well as on two healthy control populations. Thus the study design comprises the opportunity of long term correlations in a well-studied PD cohort. We have shown that genetic factors contribute in the pathogenesis in our patient cohort as well as in the German PD control cohort and have determined the frequency of known mutations. Further on, we were able to elaborate a possible genetic contribution in disease progression such as development of dementia. Further studies should involve a molecular approach of defining susceptibility markers for disease progression and thus lead to a better understanding of the molecular basis of the disease. The aim of the study of genetics in PD should be to define therapeutic opportunities based on the molecular pathogenesis of the disease.

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