Mathias Toft

Genetic studies of LRRK2 and PINK1 in Parkinson's disease

Thesis for the degree of philosophiae doctor

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«If the clinician, as observer wishes to see things as they really are, he must make a tabula rasa of his mind and proceed without any preconceived notions whatever.»

Jean-Martin Charcot

Contents

Acknowledgements

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Oslo, September 2006.

Mathias Toft

List of papers

Paper I

Kachergus J, Mata IF, Hulihan M, Taylor JP, Lincoln S, Aasly J, Gibson JM, Ross OA, Lynch T, Wiley J, Payami H, Nutt J, Maraganore DM, Czyzewski K, Styczynska M, Wszolek ZK, Farrer MJ, Toft M. Identification of a novel *LRRK2* mutation linked to autosomal dominant parkinsonism: Evidence for a common founder across European populations. *Am J Hum Genet* 2005; 76 (4): 672-680.

Paper II

Aasly JO, Toft M, Mata IF, Kachergus J, Hulihan M, White LR and Farrer M. Clinical features of *LRRK2*-associated Parkinson's disease in Central Norway. *Ann Neurol* 2005; 57 (5): 762-765.

Paper III

Toft M, Sando SB, Melquist M, Ross OA, White LR, Aasly JO, Farrer MJ. *LRRK2* mutations are not common in Alzheimer's disease. *Mech Ageing and Development* 2005; 126 (11): 1201-1205.

Paper IV

Ross OA, Toft M, Whittle AJ, Johnson JL, Papapetropoulos S, Mash DC, Litvan I, Gordon MF, Wszolek ZK, Farrer MJ, Dickson DW. Lrrk2 and Lewy body disease. *Ann Neurol* 2006; 59 (2): 88-393.

Paper V

Toft M, Myhre R, Pielsticker L, White LR, Aasly JO, Farrer MJ. *PINK1* mutation heterozygosity and the risk for Parkinson's disease. *J Neurol Neurosurg Psychiatry;* in press.

Summary in English

Background and objectives

Parkinson's disease (PD) is a common neurodegenerative disorder affecting 1% of the elderly. The disease causes a significant burden of illness and cost to society. The causes of PD have remained unknown, and the influence of genetic factors used to be controversial. In 2004, several mutations were identified in familial PD within two genes: *PINK1* and the novel gene *LRRK2*. The aims of this thesis were to further investigate genetic, clinical and pathological aspects of these genes in PD and other neurodegenerative disorders causing parkinsonism. Five papers based on data from studies of these genes are included in this thesis.

Methods

- DNA from probands of families with autosomal dominant parkinsonism were sequenced to identify novel mutations in the *LRRK2* gene. After the identification of a novel heterozygous *LRRK2* mutation, we assessed the frequency of this mutation in a total of 248 families from different populations. We also screened samples of patients with idiopathic PD from three populations (Norway, Ireland, and Poland). Family members of mutation carriers were examined, and analyses of segregation, mutation haplotypes and penetrance were performed (Paper I).

- A clinicogenetic study of PD in Central Norway was initiated several years ago at the Department of Neurology, St. Olav's University Hospital in Trondheim. We screened 435 Norwegian patients diagnosed with PD and 519 control subjects from this study for the presence of seven known *LRRK2* mutations. The clinical presentation of disease was studied in patients with mutations (Paper II).

- A series of 242 patients from a clinicogenetic study of dementia in Central Norway (Trønderbrain) were screened for the presence of seven known pathogenic mutations previously reported in the *LRRK2* gene (Paper III).

- We examined several brain banks for cases with clinical or pathological features of parkinsonian disorders. DNA was obtained from frozen brain tissue of cases with parkinsonism, other neurodegenerative disorders and controls (total n=1584) and genotyped for the exon 41 *LRRK2* g.6055G>A (G2019S) mutation. Available medical records of mutation carriers were reviewed and neuropathological examination was performed (Paper IV).

- Comprehensive *PINK1* mutation analysis was performed in a total of 131 patients from Norway with early-onset parkinsonism (onset =50 years) or familial late-onset PD. Mutations identified were examined in 350 Norwegian control individuals (Paper V).

Results

- We identified a novel heterozygous *LRRK2* g.6055G>A mutation (G2019S). Seven of 248 families with autosomal dominant parkinsonism (2.8%) and six of 806 patients with idiopathic PD (0.7%) carried this mutation. All patients with this mutation shared an ancestral haplotype, indicative of a common founder. The mutation segregates with disease (multipoint LOD score 2.41). Penetrance is age dependent, increasing from 17% at age 50 years to 85% at age 70 years (Paper I).

- Ten Norwegian PD patients were found to be heterozygote carriers of the Lrrk2 G2019S mutation. The clinical features included asymmetric resting tremor, bradykinesia, and rigidity with a good response to levodopa and could not be distinguished from idiopathic Parkinson's disease. No Parkinson's disease patient carried any of the other *LRRK2* mutations (Paper II). We did not identify *LRRK2* mutations in our series of dementia patients (Paper III).

- Lrrk2 G2019S was found in 2% (n=8) of the pathologically confirmed PD/Lewy body disease (LBD) cases (n=405). Neuropathological examination showed typical LBD in all cases (Paper IV).

- Heterozygous missense mutations in *PINK1* were found in three of 131 patients; homozygous or compound heterozygous mutations were not identified. A parkinsonian phenotype, with asymmetric onset and without atypical features, characterised these patients clinically (Paper V).

Conclusions

We identified a novel mutation in the *LRRK2* gene, g.6055G>A (G2019S). This mutation is a relatively common cause of both familial and sporadic PD, and it is found in a number of populations from North America and Europe, including Norway. This specific mutation is today the most prevalent known cause of PD, but seems to be rare in other neurodegenerative disorders.

Clinically, patients with the Lrrk2 G2019S substitution present with a levodopa–responsive parkinsonian syndrome with asymmetric resting tremor, bradykinesia, and rigidity. Both clinically and pathologically *LRRK2*-associated PD appears to be indistinguishable from idiopathic disease.

PINK1 mutations were rare in our Norwegian population, but heterozygote mutation carriers might be at increased risk for disease.

Summary in Norwegian

Bakgrunn og målsetninger

Parkinsons sykdom er en relativt vanlig nevrodegenerativ sykdom som rammer 1% av den eldre befolkningen. Sykdommen forårsaker vesentlige plager for pasientene og betydelige kostnader for samfunnet. Årsakene til Parkinsons sykdom har vært ukjente og hvorvidt genetiske faktorer medvirker har vært omstridt. I 2004 ble mutasjoner funnet hos pasienter med familiær Parkinsons sykdom i to gener: *PINK1* og det nye genet *LRRK2.* Målsetningen med denne avhandlingen var å videre undersøke genetiske, kliniske og patologiske aspekter av disse to genene ved Parkinsons sykdom og andre nevrodegenerative sykdommer som forårsaker parkinsonisme. Fem vitenskapelige arbeider basert på data fra studier av disse gene inngår i avhandlingen

Metoder

- DNA fra pasienter med autosomal dominant parkinsonisme ble sekvensert for å identifisere nye mutasjoner i *LRRK2*-genet. Etter at en ny heterozygot *LRRK2*-mutasjon ble funnet, undersøkte vi forekomsten av denne mutasjonen i totalt 248 familier fra ulike land. Vi undersøkte også prøver fra pasienter med idiopatisk Parkinsons sykdom fra tre europeiske land (Norge, Irland og Polen). Familiemedlemmer av mutasjonsbærere ble undersøkt og vi utførte analyser av segregasjon, haplotyper og penetranse av mutasjonen (Artikkel I).

- For flere år siden startet en klinisk og genetisk studie av Parkinsons sykdom i Midt-Norge ved St. Olavs Hospital i Trondheim. Vi undersøkte forekomsten av 7 mutasjoner i *LRRK2*-genet hos 435 norske pasienter diagnostisert med Parkinsons sykdom og 519 kontroller fra denne studien. Vi studerte de kliniske kjennetegnene ved sykdommen hos mutasjonsbærere (Artikkel II).

- 242 pasienter ble rekruttert fra en studie av demens i Midt-Norge (Trønderbrain) og undersøkt for forekomsten av syv kjente patogene mutasjoner som tidligere var beskrevet i *LRRK2*-genet (Artikkel III).

- Vi undersøkte flere hjernebanker for pasienter med kliniske eller patologiske tegn til parkinsonistiske sykdommer. DNA fra frossent hjernevev av avdøde pasienter med parkinsonisme, andre nevrodegenerative sykdommer og kontroller (totalt n=1584) ble genotypet for forekomst av g.6055G>A (G2019S) mutasjonen i ekson 41 av *LRRK2*-genet. Vi gjennomgikk tilgjengelige journalopplysninger av mutasjonsbærere og utførte nevropatologiske undersøkelser (Artikkel IV).

- Omfattende mutasjonsanalyser av *PINK1*-genet ble utført i totalt 131 pasienter fra Norge med parkinsonisme med sykdomsdebut =50 år eller familiær Parkinsons sykdom. Identifiserte mutasjoner ble undersøkt i 350 norske kontroller (Artikkel V).

Resultater

- Vi identifiserte en ny heterozygot *LRRK2* g.6055G>A (G2019S) mutasjon. Syv av 248 familier med autosomal dominant parkinsonisme (2.8%) og seks av 806 pasienter med sporadisk Parkinsons sykdom (0.7%) var bærere av denne mutasjonen. Alle disse pasientene deler en felles haplotype, noe som indikerer felles opphav. Mutasjonen segregerer med sykdommen i familiene (multipoint LOD-score 2.41). Penetransen er aldersavhengig og øker fra 17% ved 50-års alder til 85% ved 70-års alder (Artikkel I).

- Totalt ti norske pasienter med Parkinsons sykdom var heterozygote bærere av G2019S-mutasjonen i *LRRK2*-genet. Klinisk presenterte sykdommen seg med asymmetrisk hviletremor, bradykinesi og rigiditet med god effekt av levodopa-behandling, og symptomene skilte seg ikke fra idiopatisk Parkinsons sykdom. Ingen av pasientene var bærere av noen av de andre undersøkte mutasjonene (Artikkel II). Vi fant ingen mutasjoner i *LRRK2*-genet i vår studie av pasienter med demens (Artikkel III).

- *LRRK2* G2019S-mutasjonen ble funnet i 2% (n=8) av de patologisk verifiserte tilfellene av Parkinsons sykdom/lewylegemesykdom (n=405). Nevropatologisk undersøkelse viste typisk lewylegemesykdom i alle tilfellene (Artikkel IV).

- Vi identifiserte heterozygote mutasjoner i *PINK1*-genet hos tre av 131 pasienter, ingen av pasientene hadde homozygote mutasjoner. Et parkinsonistisk kliniske bilde med asymmetrisk start uten atypiske symptomer var karakteristisk hos disse pasientene (Artikkel V).

Konklusjoner

Vi identifiserte en ny mutasjon i *LRRK2*-genet som fører til en G2019S-endring av proteinstrukturen. Denne mutasjonen er en relativt vanlig årsak til både familiær og sporadisk Parkinsons sykdom. Mutasjonen ble funnet i flere populasjoner fra både Nord-Amerika og Europa, inkludert Norge. Mutasjonen er i dag den vanligste kjente årsaken til Parkinsons sykdom i verden, men sjelden i andre nevrodegenerative sykdommer. Studien viser at genetiske faktorer er viktigere for sykdomsutviklingen enn tidligere antatt.

Klinisk presenter pasienter med Lrrk2 G2019S-mutasjonen et levodopa-responsivt parkinsonistisk syndrom med asymmetrisk hviletremor, bradykinesi og rigiditet. Både klinisk og patologisk synes *LRRK2*-assosiert Parkinsons sykdom å være identisk med idiopatisk sykdom***.***

Mutasjoner i *PINK1*-genet er sjeldne i Norge, men heterozygote mutasjonsbærere har muligens øket risiko for utvikling av Parkinsons sykdom.

Abbreviations and definitions

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
DLB	Dementia with Lewy bodies
EOP	Early-onset parkinsonism
Genotype	The particular set of alleles that an individual has at a given region of the genome.
GTP	Guanosine triphosphate
Haplotype	A particular combination of alleles that are closely linked on a chromosome.
LBD	Lewy body disease
LOD-score	Logarithm of odds-score
LRRK2	Leucine-rich repeat kinase 2
MAPT	Microtubule-associated protein tau
MPTP	N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSA	Multiple system atrophy
Mutation	An alteration in a genome compared to some reference state. A mutation does not have to have harmful effects.
PD	Parkinson's disease
PET	Positron emission tomography
Phenotype	The observable properties and characteristics of an individual or a locus
PINK1	PTEN-induced kinase 1
Polymorphism	A region on the genome that varies between individual members of a population.
PSP	Progressive supranuclear palsy
SNP	Single nucleotide polymorphism
SPECT	Single photon emission computer tomography

1. General introduction

1.1 Historical background

> *"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 senses and intellects being uninjured."*

This was the definition of paralysis agitans given by James Parkinson (1755-1824) in his classical publication *An essay on the shaking palsy* (1). Parkinson noted the occurrence of tremor, alterations of gait and posture, hypophonia, dysgraphia, and sialorrhea.

Figure 1. Jean-Martin Charcot (1825 – 1893)

For decades Parkinson's work went largely unrecognized until Jean-Martin Charcot (Figure 1) further defined the syndrome by adding rigidity to the symptoms, and in tribute named the disorder *maladie de Parkinson* (Parkinson's disease, PD). In his later years, Charcot was interested in the idea of disorders running in families, and his students also studied the heritability of PD.

For a long time PD remained an untreatable disorder with devastating consequences for the patients. The key event leading to the development of effective treatment was the discovery by Ehringer and Hornykiewicz of striatal dopamine deficiency in brains of PD patients (2). For the first time levels of a specific neurotransmitter correlated with a disease of the brain.

Subsequently, levodopa was tried in PD patients, but throughout most of the 1960s the results were inconsistent. In 1967, questions about the effectiveness of levodopa in PD were finally set aside when Cotzias and colleagues reported dramatic improvement in PD patients with oral administration of levodopa in increasing amounts over long periods (3).

Dopamine replacement therapy allows remarkable long-term symptomatic control over the motor features of PD. Other existing treatments, including deep brain surgery, can control the motor complications associated with chronic levodopa treatment. However, the patients' quality of life continues to deteriorate as a consequence of the so-called "non-dopaminergic" clinical manifestations: gait and equilibrium difficulties, autonomic dysfunction, depression and cognitive impairment (4).

Thus, the present challenge is to increase the biological understanding of the neurodegenerative process, so that new therapies slowing and halting disease progression can be developed. Studies of genetic defects causing parkinsonism, and of patients affected by these genetic disorders, have identified key proteins and pathways involved in neuronal cell death. Genetic insights have provided the rationale for new strategies for prevention and therapy. The primary aim of this thesis was to identify genetic causes of Parkinson's disease and to study the clinical and pathological features associated with it.

1.2 Definitions

Parkinsonism

Parkinsonism is a clinical syndrome characterized by the cardinal motor signs: bradykinesia, resting tremor, muscle rigidity, and postural instability. A large number of neurodegenerative and other disorders of the central nervous system can present with parkinsonism.

Parkinson's disease

A diagnosis of PD is based on the clinical identification of some combination of the mentioned cardinal motor signs, asymmetry of disease onset, response upon dopaminergic treatment, and absence of atypical symptoms. In addition, a disease causing parkinsonism and secondary causes of parkinsonism should be absent.

There is no definite biomarker for PD. Routine blood tests, structural imaging of the central nervous system and other paraclinical tests are mainly used to exclude another etiology for the parkinsonian syndrome. Functional imaging, such as SPECT and PET, can directly assess neurotransmitter activity in the nigrostriatal dopaminergic system. Most causes of parkinsonism are associated with reduced striatal tracer uptake compared with normal aging. PD can, to some extent, be differentiated from other causes of parkinsonism because it is associated with particularly low levels of tracer uptake in the putamen. PD has however so far remained a clinical diagnosis.

Several groups have therefore proposed diagnostic criteria for a diagnosis of PD, to reliably distinguish PD from other conditions with parkinsonian features. In Papers I, II and V, the criteria proposed by Gelb and colleagues were used. Three levels of diagnostic confidence are differentiated: definite, probable and possible. The diagnoses of possible and probable PD are based on clinical criteria alone, whereas neuropathological confirmation is required for the diagnosis of definite PD (Table 1) (5).

Familial and sporadic PD

In this thesis, the term PD describes any patient fulfilling these diagnostic criteria, including patients with a family history of parkinsonism. In the literature, patients with a clinical syndrome indistinguishable from typical PD caused by known genetic mutations have been referred to using the terms PD and parkinsonism. It could be argued that patients affected by parkinsonism with a known etiology should not be referred to as having PD, and that this term should be reserved for idiopathic cases. However, from a clinical point of view these patients can fulfill all proposed criteria. Patients with an unknown cause of PD are referred to as having idiopathic PD.

Sporadic PD is in this thesis defined as PD in a patient without any first or second degree relative having a known diagnosis of PD.

Familial PD is defined as PD in a patient with at least one first or second degree relative with PD.

Table 1. Diagnostic criteria for PD (from Gelb et al., ref. 5)

Grouping of clinical features according to diagnostic utility
Group A features: characteristic of PD
Resting tremor
Bradykinesia
Rigidity
Asymmetric onset
Group B features: suggestive of alternative diagnoses
Features unusual early in the clinical course
- Prominent postural instability
- Freezing phenomena
- Hallucinations
- Dementia preceding motor symptoms or in the first year
Supranuclear gaze palsy or slowing of vertical saccades
Severe, symptomatic dysautonomia
Documentation of a condition known to produce parkinsonism plausibly connected to the symptoms

Proposed diagnostic criteria for Parkinson's disease
Criteria for POSSIBLE diagnosis of PD
- At least 2 of 4 features in Group A, at least one of these is tremor or bradykinesia
- None of the features in Group B (or symptoms for less than 3 years)
- Response to dopaminergic treatment or not had adequate trial
Criteria for PROBABLE diagnosis of PD
- At least 3 of 4 features in Group A
- None of the features in Group B
- Response to dopaminergic treatment
Criteria for DEFINITE diagnosis of PD
- All criteria for POSSIBLE PD are met
- Histopathological confirmation

Autosomal dominant and recessive PD

Autosomal dominant inheritance refers to genetic conditions that occur when mutations are present in one allele of a given gene. Families with two or more members affected by PD in at least two consecutive generations are in Paper I considered to be consistent with an autosomal dominant pattern of inheritance.

Autosomal recessive inheritance refers to genetic conditions that occur only when mutations are present in both alleles of a given gene. In Paper V, 20 patients with a family history consistent with recessive inheritance were included. This was broadly defined by the presence of parkinsonism in siblings and/or first degree cousins, without evidence of affected parents or offspring.

Early-onset parkinsonism

In this thesis a patient affected by parkinsonism at 50 years of age or earlier is considered to have early-onset parkinsonism (EOP).

1.3 Epidemiology

The prevalence of PD has been estimated in several studies, and most of them have found prevalence figures between 100 and 150 per 100.000 inhabitants (6). In a community study from the County of Rogaland in Norway the total age-adjusted prevalence rate was 102 per 100,000. Men are somewhat more likely to develop the disorder. In the mentioned study, sex specific age-adjusted prevalence rates were 121 per 100,000 men and 90 per 100,000 women (6).

Mean age of onset is 58 to 62 years in most reports. The frequency of PD increases with age, which is the strongest risk factor disease development . In a study from Rotterdam, PD affected more than 1% of the population older than 55 years of age (7). Hence, PD is a prevalent disease among the elderly.

1.4 Differential diagnosis of parkinsonism

The most common cause of parkinsonism is PD, but parkinsonism is also frequent in a large number of other neurodegenerative disorders (Table 2). Symptomatic parkinsonism occurs secondary to the use of drugs with antidopaminergic effects, and also in vascular, toxic, metabolic, infectious, and post-infectious disorders.

The clinical diagnostic accuracy of PD can be improved with the use of published and validated criteria (5). However, because of the overlapping clinical features of parkinsonian disorders, histopathologic confirmation is still required for the definite diagnosis of PD and other parkinsonian disorders (8, 9).

Table 2. Neurodegenerative disorders manifesting parkinsonism

Synucleinopathies
Lewy body disorders
Parkinson's disease
Dementia with Lewy bodies
Pure autonomic failure
Glial inclusion body disorders
Multiple system atrophy
Other synucleinopathies
Pantothenate kinase associated neurodegeneration
Pallidonigroluysian atrophy
Tauopathies
Progessive supranuclear palsy
Corticobasal degeneration
Frontotemporal dementia with parkinsonism
Alzheimer's disease
Postencephalitic parkinsonism
Guam amyotrophic lateral sclerosis/parkinsonism dementia complex
Other neurodegenerations
Spinocerebellar ataxia 2
Spinocerebellar ataxia 3
Dentatopallidoluysian dystrophy
X-linked dystonia-parkinsonism

1.5 Pathology of Parkinson's disease

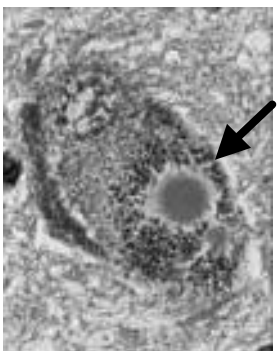

Figure 2. A Lewy body within a neuron in the substantia nigra.

The principal neuropathological changes in PD are depigmentation, loss of cells and gliosis in the *substantia nigra,* with formation of Lewy neuritis and Lewy bodies within many of the remaining neurons (Figure 2). The nigral damage is accompanied by pathology in the *locus ceruleus*, dorsal motor nucleus of the vagus nerve, *nucleus basalis* of Meynert, and the ventral tegmental area of the midbrian as well as other subcortical nuclei. In more advanced stages lesions reach the neocortex (9).

The pathological term Lewy body disease (LBD) includes the clinical diagnoses PD with and without dementia, as well as dementia with Lewy bodies (DLB). DLB exhibits a clinical phenotype apparently different from PD, but the morphology of the Lewy neurites and Lewy bodies, the characteristics of the vulnerable neuronal types, and the distribution of affected subcortical nuclei and cortical areas closely overlap with those of PD (10). Brainstem predominant, limbic (transitional) and neocortical LBD are distinguished on neuropathological examination.

The incidence of Lewy bodies in the brains of asymptomatic individuals increases with advancing age. Lewy bodies also occur in 10 to 40% of individuals with AD and in some other neurodegenerative disorders (5). This indicates that Lewy bodies might not represent specific underlying pathological mechanisms. Similarly, many cases of PD with Lewy bodies have concurrent pathologic findings typical for AD.

Lewy bodies, first described by Friedrich Heinrich Lewy in 1912, are eosinophilic cytoplasmic fibrillar aggregates containing α-synuclein and various other proteins and are found in affected brain regions. α-synuclein aggregation is a pathologic feature common to sporadic and inherited forms of PD, as well as to other neurodegenerative disorders, and these disorders have collectively been called synucleinopathies (Table 2).

Other forms of parkinsonism are characterized neuropathologically by prominent intracellular accumulations of abnormal filaments of the microtubule-associated protein tau, known collectively as neurodegenerative tauopathies (Table 2). Mutations in this gene (*MAPT*) are found in families with frontotemporal dementia with parkinsonism (11). Common variants in the *MAPT* gene are associated with progressive supranuclear palsy (PSP) (12), and possibly also with corticobasal degeneration (13). More intriguingly, in a study not included in this thesis we found an association between *MAPT* haplotypes and PD, demonstrating a possible link between PD and other causes of parkinsonism (14).

1.5 Alzheimer's disease

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It is characterized clinically by a gradual onset and progression of memory loss. Parkinsonism can be a part of the clinical presentation. At postmortem examination there is presence of two types of neuropathological inclusions: neurofibrillary tangles and senile plaques. Neurofibrillary tangles are composed of paired helical filaments of hyperphosphorylated tau protein, whereas the main proteinaceous component of senile plaques is ß-amyloid.

Clinical diagnostic criteria have been developed, increasing the accuracy of the diagnosis relative to neuropathologic examination. The most frequently used criteria for the diagnosis of AD are those of the NINCDS-ADRDA (15). These criteria classify AD based on degree of certainty and whether AD is associated with other disease processes.

Currently, there are four genes that are implicated in risk for familial AD. Mutations in the genes that encode ß-amyloid precursor protein, presenilin-1, and presenilin-2 cause the rare early-onset form of familial AD. The fourth gene, which encodes apolipoprotein E, is a major risk factor in both early-onset (onset before 65 years) and late-onset (onset after 65 years) AD. However, these four genes together may account for less than half the genetic variance in AD, and possibly several other genes remain to be identified (16).

1.7 Heredity and familial aggregation

Leroux and Lhirondel, two of Charcot's students at the *Hôpital de la Salpêtrière* in Paris, were probably the first to record a familial component to PD, stating that "a true cause of paralysis agitans, and maybe the only true cause, is heredity" (17). Several other reports in early European literature also described hereditary parkinsonism (18).

Henry Mjönes, who studied familial parkinsonism in Sweden in the 1940's, was the first to use a systematic genetic-statistical approach. In his thesis, he proposed that PD was inherited in an autosomal dominant fashion with reduced

penetrance (19) (Figure 3). Although other reports provided additional evidence that genetic factors may be important in the genesis of PD (20), the role of genetics remained controversial.

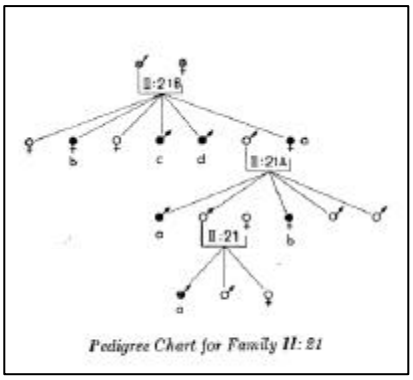

Figure 3. The pedigree shown is one of the families studied by Henry Mjönes and is an example of the proposed autosomal dominant inheritance model. Individuals affected by PD are denoted with a blackened circle.

A family history of PD is second only to age as a predictor of increased risk of the disease (21, 22). Numerous studies have investigated familial aggregation of PD and the majority has reported higher frequency of PD among relatives of probands compared to relatives of control individuals. The estimate of relative risk has varied from 2.3 to 14.6 (23). A recent study, which used a family study method assessing relatives individually, confirmed that relatives of patients with younger disease onset (<67 years) were at increased risk (24).

Concordance rates of disease in monozygotic and dizygotic twin pairs have traditionally been used to measure the genetic contribution to any condition. Large cross-sectional twin studies have identified significant differences in concordance rates between monozygotic and dizygotic twins in early-onset PD (age at onset <50 years), but not in late-onset disease (25, 26). However, even these large and well-designed twin studies are probably underpowered to detect incompletely penetrant mutations (27).

Longitudinal twin studies using ^{18}F-dopa PET have been used to highlight clinically pre-symptomatic dopaminergic loss. Results suggest 75% disease concordance in monozygotic twins, versus 22% concordance in dizygotic pairs, regardless of age at onset (28). Based on the combination of clinical data and genealogical records, significant clustering for late onset PD was shown in Iceland with familial aggregation extending beyond the nuclear family (29).

In total, these data suggest that the contribution of genetics to parkinsonism may be greater than previously appreciated. On the other hand, most patients do not have a clear family history of disease, probably because either the causative genes have low penetrance, or the disorder is a result of a combination of genetic predisposition, environmental exposure and stochastic factors.

1.8 Parkinson's disease and the environment

Environmental factors were long thought to be the predominant cause of PD (30). The epidemic of *encephalitis lethargica* in the beginning of the 20th century left large numbers of survivors with neurologic sequelae, including a form of progressive parkinsonism. Subsequently, infectious agents were suspected to be environmental factors causing PD (31). However, PD does not generally present in clusters or epidemics, making this hypothesis an unlikely explanation for the majority of cases.

In the early 1980s, drug addicts mistakenly synthesized and injected themselves with N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and developed a levodopa-responsive parkinsonian syndrome (32). MPTP causes selective degeneration of the nigrostriatal pathway by inhibition of mitochondrial complex I (33), and following this discovery other toxins affecting the mitochondrion, such as epoxymycin and rotenone, were studied as potential environmental causes of PD.

A large number of environmental agents ranging from rural living, industrial toxins and heavy metals have been examined, although no conclusive link has yet been identified (30). Smoking has an inverse association with PD, whereas studies of other environmental agents have been consistently inconsistent. In conclusion, no environmental factor has so far been established as definitively associated with PD.

1.9 Genetics of familial parkinsonism

A major breakthrough in recent years has been the mapping of a number of loci linked to familial parkinsonism and the cloning of several genes causing monogenic forms of the syndrome (Table 3). In addition, at least 5 other genetic disorders have phenotypic overlap with PD (Table 4). These diseases should be thought of separately from the *PARK* loci, as they rarely present clinically with parkinsonism only.

Table 3. Familial parkinsonism with reported mutations/loci

Locus	*Chromosome*	*Gene*	*Clinical phenotype*
Autosomal dominant			
PARK1/4	4q21	*α-synuclein*	Early onset PD and DLB
PARK3	2p13	Unknown	PD
PARK5	4p14	*UCH-L1*	PD
PARK8	12q12	*LRRK2*	Predominantly PD; also dementia, PSP-like and ALS
Autosomal recessive			
PARK2	6q25-27	*Parkin*	Early-onset PD
PARK6	1p35-36	*PINK1*	Early-onset PD
PARK7	1p36	*DJ-1*	Early-onset PD
Unknown			
PARK10	1p32	Unknown	PD
PARK11	2q36-37	Unknown	PD

Despite the discovery of genetic defects in familial parkinsonism, the role of genetics in sporadic late-onset PD has remained controversial. This controversy has not only been caused by the relatively low number of patients carrying known mutations in these genes, but also by the clinical and neuropathologic differences between sporadic PD and the hereditary forms of parkinsonism. However, with the increasing number of patients identified with a genetic form of PD, this view is gradually changing.

Table 4. Genetic diseases with parkinsonism as part of the clinical spectrum

Disease	Chromosome	Gene	Clinical phenotype
SCA2	12q23-24	*ATXN2*	Ataxia, parkinsonism
SCA3	14q32	*ATXN3*	Ataxia, parkinsonism
FTDP-17	17q21-22	*MAPT, PGRN*	FTD, PD, PSP, CBD, ALS
XDP (DYT3)	Xp13.1	Unknown	Dystonia-parkinsonism
RDP (DYT12)	19q13	*ATP1A3*	Dystonia-parkinsonism

ATP1A3, Na+/K+ ATPase alpha 3 polypeptide; ATXN2, Ataxin 2; ATXN3, Ataxin 3; CBD, Corticobasal degeneration; FTP-17, Frontotemporal dementia linked to chromosome 17; MAPT, Microtubule-associated protein tau; PGRN, Progranulin; RDP, Rapid-onset dystonia-parkinsonism; SCA, Spinocerebellar ataxia; XDP, X-linked dystonia parkinsonism.

In the following section, the previously identified genes in familial parkinsonism will be briefly reviewed.

***α-synuclein* (PARK1)**

In 1996, the first locus for autosomal dominantly inherited parkinsonism was mapped to chromosome 4q21-q23 in the Contursi kindred, a large kindred of Italian descent (34). Subsequently, a missense mutation in the *α-synuclein* gene leading to an A53T amino acid substitution was identified in several families (35). Confirmation of α-synuclein's involvement in disease was provided when a second pathogenic mutation, A30P, was identified in a small German family (36). Missense mutations in the *α-synuclein* gene are rare (37). Numerous patients from families with parkinsonism have now been sequenced (38); only one additional pathogenic mutation (E46K) has been identified in a Basque family (39).

Clinically, members of the Contursi kindred (A53T) showed typical features of PD, including the cardinal motor features and a positive response to levodopa treatment (40). Other patients with the A53T substitution have presented a broader phenotype with central hypoventilation, orthostatic hypotension and myoclonus (41). Compared with idiopathic PD, disease onset in mutation carriers occurs relatively early in life, and the course from disease onset to death is rapid. Clinical symptoms of patients with A30P closely resembles

idiopathic PD (42). Carriers of the E46K mutation present severe parkinsonism with development of dementia, hallucinations and fluctuations of consciousness (39).

Genomic multiplications of the complete *α-synuclein* gene have also been linked to familial PD. Genomic triplications of the locus were identified in the large Spellman-Muenter kindred and in a Swedish-American family (43, 44). Subsequently, three French families have been identified with duplications of the normal *α-synuclein* gene (45, 46). A large number of PD patients have now been screened for multiplications, demonstrating that also this is a rare disease mechanism (47-49).

A direct relationship between *α-synuclein* gene dosage, expression and the age of disease onset, progression and phenotypic severity has been observed (44). Genomic triplication of the gene causes a rapidly progressive form of parkinsonism characterized by young age at onset, weight loss, followed later by dementia and early death (18, 43). In contrast, the clinical phenotype of families with a duplication of the wild-type gene resembles idiopathic PD with late age at onset, slower disease progression and without early development of dementia (45, 46).

A possible role of *α-synuclein* in the pathogenesis of sporadic PD was suggested after the finding of the α-synuclein protein as a major component of Lewy bodies (50). Antibodies raised against α-synuclein stain Lewy body inclusions in surviving neurons of the *substantia nigra* in both familial and sporadic PD.

The three substitutions alter the properties of the α-synuclein protein, leading to an increased propensity of the protein to aggregate (51, 52). This process is thought to be a crucial step in formation of the Lewy bodies, and therefore in the molecular pathogenesis of the disease. The presence of α-synuclein containing Lewy bodies in the absence of coding mutations in sporadic PD suggests that other α-synuclein modifications or additional interacting genes may be contributing to sporadic PD.

***Parkin* (PARK2)**

Mutations in the *parkin* gene were first described in consanguineous Japanese families with autosomal recessive juvenile parkinsonism (53). Numerous mutations (>100) including exonic deletions, insertions, and point mutations have been observed in patients of all ethnic backgrounds (reviewed by (54). *Parkin*-associated early-onset PD (<45 years) is relatively common. In a study of individuals from different European populations, this gene caused 49% of familial early-onset PD and 18% of sporadic early-onset disease (55). Similar mutation frequencies have also been found in several other studies (56, 57).

However, the genetic epidemiology of *parkin* is complex. The frequency of mutations in sporadic patients decreases significantly with increasing age at onset, and *parkin* mutations are rare in patients with late-onset PD (>50 years) (55, 58). The role of heterozygous mutations as risk factor for PD is still unclear, as the results in the literature are contradictory. Evidence for dominant *parkin*-proven familial disease has been published (59, 60), and one study found reduced ^{18}F-dopa uptake in clinically asymptomatic carriers in a family with *parkin* mutations (61). Intriguingly, mutation carriers in other families do not show signs of parkinsonism (62), and in North American community based late-onset PD, the carrier frequency of heterozygous *parkin* mutations is ~3%, similar to that in controls (63).

The clinical phenotype most commonly resembles sporadic disease, but a number of clinical features are associated with *parkin* disease. Symmetrical involvement, dystonia and hyperreflexia at onset is more common (55, 64). The disease course seems to be relatively benign with slow disease progression, sleep benefit and good response to levodopa, but complicated with early motor fluctuations and development of dyskinesias (65). Pyramidal signs, cerebellar features and psychiatric disease have been reported, though dementia seems to be rare (64).

Neuropathological studies of patients with *parkin* mutations with homozygous exonic deletions show selective cell loss of the nigrostriatal tract and *locus ceruleus*, with a remarkable absence of Lewy bodies (66, 67). In contrast, Lewy

body pathology or neurofibrillary tangles have been identified *post-mortem* in compound heterozygous cases with possible partial parkin function (60, 68).

Cell loss in the *substantia nigra* in *parkin*-associated PD appears to be caused by a loss of function of the parkin protein. Functionally, the parkin protein is a member of a family of E3 protein ligases responsible for the transfer of activated ubiquitin molecules to a substrate (69). Numerous potential substrates have been nominated (54). Depending on the mutation, mutants have either reduced or absent enzymatic function, which may help explain the conflicting data on disease susceptibility and outcome in carriers (70). Polymeric ubiquination of a protein typically acts as a signal for its subsequent proteasomal degradation. In this process waste, damaged or misfolded proteins are tagged for destruction by the proteasome. There is also some evidence indicating that inhibition of the ubiquitin-proteasome might also be responsible for degeneration of the nigrostriatal pathway in idiopathic PD (71).

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1; PARK 5)

By sequencing of 72 families with PD, Leroy and colleagues identified a single missense mutation g.277C>G (I93M) in the *UCH-L1* gene in two German siblings (72). In both patients the clinical syndrome was typical for PD, with disease onset at a mean age of 50 years, and a beneficial response to levodopa replacement therapy. There are no radiological or neuropathological reports available on this family. The significance of these findings is however uncertain, as no other families with mutations in this gene have been found to date (73, 74). A common coding polymorphism in the *UCH-L1* gene leading to a S18Y substitution has been identified; the Y18 variant was reported to be inversely associated with PD in a dose-dependent manner (75). However, this association has been questioned by a recent case-control study and meta-analysis (76).

UCH-L1 is one of the most abundant proteins in the brain and immunofluorescence studies of Lewy bodies are positive for UCH-L1 protein, which possibly implicates it either directly or indirectly with the development of

PD (77). The protein is also functionally involved in the ubiquitin-dependent proteolytic pathway; hence *UCH-L1* is a good candidate gene for PD.

DJ-1 (PARK7)

In 2003, mutations in the *DJ-1* gene were identified in two consanguineous families with early-onset autosomal-recessive parkinsonism originating from the Netherlands and Italy (78). Pathogenic *DJ-1* mutations in early-onset PD are rare and the mutation frequency in early-onset PD has been estimated at approximately 1% (79). Several other studies have failed to identify *DJ-1* alterations in PD patients originating from different populations (80-82).

Clinically, patients with *DJ-1* mutations have asymmetric symptoms with slow progression and sustained response to levodopa treatment. Age of onset is typically between 20 and 40 years. Focal dystonia and psychiatric co-morbidity have been reported (83, 84). The neuropathology associated with *DJ-1* parkinsonism is still unknown. Functional neuroimaging of *DJ-1* homozygous mutation carriers showed a decreased ^{18}F-dopa uptake concordant with typical PD. Clinically unaffected heterozygous mutation carriers had normal ^{18}F-dopa metabolism. This indicates that heterozygosity is not a risk factor for PD and that a nearly complete loss of DJ-1 protein function is necessary to cause disease (85).

The function of DJ-1 is unknown, but it is an abundant protein dimer in brain, mainly expressed in astrocytes (86). An acidic isoform accumulates after oxidative stress, indicating that DJ-1 limits cellular toxicity (87). Oxidative conditions induce a modification of DJ-1, supporting the hypothesis that DJ-1 is an oxidative stress sensor within cells (88). Studies of the dopaminergic system in DJ-1-deficient mice have suggested an essential role for DJ-1 in dopaminergic physiology and D2-receptor mediated functions (89). The DJ-1 protein is localized to mitochondria, at least in a proportion of transfected cells, suggesting that DJ-1 can be targeted to the mitochondrion under certain conditions and protect against neuronal death (78, 90). Thus, DJ-1 further indicates a link between mitochondrial impairment and the pathogenesis of Parkinson's disease.

1.6 PTEN-induced kinase 1 (PINK1)

In 2004 Valente and colleagues identified mutations in the *PTEN-induced kinase 1* (*PINK1*) gene in three families with autosomal recessive EOP previously linked to the *PARK6* locus on chromosome 1p35-36 (91). One homozygous truncating mutation was found in two consanguineous Italian families, whereas a homozygote missense mutation at a highly conserved amino acid was found in a third consanguineous family of Spanish origin.

Other *PINK1* mutations have now been identified in families from different European, Asian, African and North American populations (92-95). Ibanez and colleagues studied 177 autosomal recessive PD families with ages at onset =60 years and found homozygous or compound heterozygous mutations in seven families. This study suggested that *PINK1* is the second most frequent causative gene in EOP (96). *PINK1* mutations have also been found as a relatively rare cause of sporadic early-onset PD (97-99).

The clinical picture of *PINK1* associated disease was first reported to be characterized by a typical parkinsonian phenotype with asymmetric onset and rare occurrence of atypical features (97). Slow progression of disease, early onset of levodopa-induced dyskinesias and sustained response to dopaminergic treatment is common (100). *PINK1* mutations cause PD with early onset, and patients reported have mainly presented with symptoms before the age of 50. The median age at onset has been reported to be around 35 years (96).

Recent studies have indicated that the phenotype associated with *PINK1* mutations might be broader than first reported. Compared to patients without mutations in *PINK1* or *parkin*, *PINK1* mutation carriers more frequently presented with dystonia at onset and hyperreflexia in the lower limbs. In addition, psychiatric disturbances has been found in a number of patients (92, 94, 96).

The neuropathological substrate of *PINK1* associated PD is unknown, as no reports have been published. However, a ^{18}F-dopa PET study showed a different pattern of nigrostriatal dopaminergic dysfunction in *PARK6*-linked PD than idiopathic disease, indicating different neuropathological features (101).

The *PINK1* gene has 8 exons and encodes a serine/threonine kinase localized to the mitochondrion. Little is known about protein function, but it may protect neurons from stress-induced mitochondrial dysfunction (91). Specific mutations have been shown to impair protein folding/half-life and kinase activity *ex vivo* (102). Recent reports have indicated genetic interactions between *PINK1* and *parkin*. Loss of *PINK1* in *Drosophila melanogaster* models lead to defects in mitochondrial function with muscle and dopaminergic neuron degeneration that can be rescued by *parkin* (103-105). Hence, the two genes appear to function in a common pathway.

1.11 Leucine-rich repeat kinase 2 (LRRK2)

In 2002, Funayama and colleagues performed a genome-wide linkage analysis of a Japanese family with autosomal dominant parkinsonism (106). In this family, also known as the Sagamihara kindred, members presented with clinical features that may not be distinguished from sporadic late-onset PD (107). The clinical symptoms responded well to levodopa, and mean age at symptom onset was 51 years. Neuropathologic examinations in 4 members of the kindred showed pure nigral degeneration without any identified Lewy bodies.

Parametric 2-point linkage analysis generated a highly significant logarithm of odds (LOD) score of 4.32 at the marker D12S345. Haplotype analysis of markers on chromosome 12 shared by affected family members defined the disease-associated haplotype to a relatively large 13.6-cM region located to 12p11-q13 (106). The chromosome 12 locus differed from previously reported regions linked to familial parkinsonism and was assigned the symbol *PARK8.*

After identification of the *PARK8* locus, linkage to this region was confirmed in a study of autosomal dominant parkinsonism in 21 families originating from

Europe and North America (108). Based on analysis of the two kindreds with the highest LOD scores in this study (Family A and Family D), the most likely disease gene location was a 3.2-cM region on chromosome 12q12. A study of 4 Basque families also found evidence for linkage of autosomal dominant PD to the *PARK8* locus, with a maximum 2-point LOD score of 3.21 (109). Combined, these studies provided evidence that the *PARK8* locus is responsible for a subset of families with autosomal dominant parkinsonism and suggested that the locus may be relatively common and occur in patients from different populations.

The existence of a gene within the *PARK8* locus associated with familial parkinsonism was finally established when the two groups identified a total of seven mutations in a novel gene, which was assigned the name *leucine-rich repeat kinase 2* (*LRRK2)* (110, 111). All mutations were located within the predicted functional domains of the novel protein and segregated with disease within the families. Clinically, most patients in these studies presented with late-onset Parkinson's disease. However, neuropathological examinations demonstrated brainstem dopaminergic degeneration accompanied by strikingly diverse pathologies.

The *LRRK2* gene is located close to the centromere on the long arm of chromosome 12, and the gene was not studied until the identification of pathogenic mutations in parkinsonian kindreds. To establish the complete cDNA sequence, the *LRRK2* gene was amplified from human brain cDNA using overlapping primers predicted by homology searches. The gene spans a genomic region of 144 Kb, with a total of 51 exons encoding a 2,527–amino acid protein (Figure 4) (111).

Using Northern blots and real-time reverse transcriptase–polymerase chain reaction methods, expression analyses have shown that the LRRK2 gene is expressed at low levels throughout the adult human brain, with slightly higher expression in putamen and substantia nigra than in other brain regions. Of other tissues examined, the gene expression is highest in lungs (110, 111).

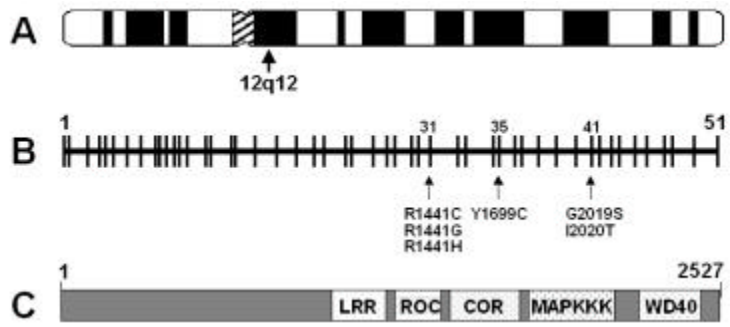

Figure 4. Chromosome 12 and the structure of the *LRRK2* gene and the Lrrk2 protein. *A*) The PARK8 locus is located on chromosome 12q12. *B*) The *LRRK2* gene has 51 exons; the localization of mutations with proven pathogenicity is noted. *C*) Pathogenic mutations are located within the functional domains. COR, C-terminal of Roc; LRR, leucine-rich repeat; MAPKKK, mitogen-activated protein kinase kinase kinase; ROC, Ras in complex proteins; WD40, WD40 repeats.

The function of the Lrrk2 protein is still largely unkown. However, *in silico* predictions and homology searches of similar proteins in other species indicate that Lrrk2 is a member of the recently defined Roco protein family. In humans, mice, and rats, members of the Roco family have five conserved functional domains (Fig. 1) (112). These multidomain proteins have been found in species ranging from mammals to metazoans and exhibit various functions.

The Lrrk2 protein has a large N- terminus ending with ankyrin and leucine-rich repeats (LRR) consisting of 12 strands of a 22– to 28–amino acid motif presented in a tandem array. The Roc (for Ras of complex proteins) domain contains a GTPase-like domain with homology to all four members of the GTPase superfamily. GTPases are small proteins that regulate a wide array of cellular processes, such as signaling, differentiation, and growth through binding and hydrolysis of guanosine triphosphate (GTP) (112).

All Roco proteins contain a novel COR (C-terminal of Roc) domain, which is about 300 to 400 amino acids long. The function of this domain is currently unknown. A kinase domain with a catalytic core common to serine and threonine and to tyrosine protein kinases is always present in this protein family. The kinase domain belongs to the MAPKKK subfamily of kinases. There is a WD40 repeat domain at the carboxylate terminus.

2. Aims of the studies

Paper I

- Sequence the *LRRK2* gene in families previously linked to the *PARK8* locus to identify novel mutations.
- After the identification of a novel G2019S mutation, we wanted to examine the mutation frequency in autosomal dominant and sporadic Parkinson's disease.
- Examine the segregation pattern and penetrance of this mutation within families.

Paper II

- Examine the presence of *LRRK2* mutations in a clinic-based sample of PD from Central Norway.
- Describe the clinical features of *LRRK2*-associated PD.

Paper III

- Examine the frequency of *LRRK2* mutations in neurodegenerative disorders causing dementia in a sample from Central Norway.

Paper IV

- Investigate the frequency of the Lrrk2 G2019S substitution in a brain bank series of cases with clinical or pathological features of parkinsonism.
- Describe the pathology associated with disease in identified cases with a *LRRK2* mutation.

Paper V

- Examine the role of mutations in the *PINK1* gene in a Norwegian series of early-onset parkinsonism and familial late-onset PD.

3. Materials

3.1 Patients and control subjects

Four of the papers in this thesis have used DNA and clinical information obtained from clinical samples of patients with neurodegenerative disorders:

PD – Trondheim

For Papers I,II and V we used a clinic-based series from Central Norway. Inclusion of patients with PD into this study has been performed since 1998. Four hundred and thirty-five patients have been clinically examined and are followed longitudinally by one neurologist (Jan O. Aasly) at the outpatient clinics of three hospitals in Central Norway (St. Olav's Hospital, Trondheim; Ålesund Hospital, Ålesund; and Helgeland Hospital in Mosjøen). A total of 403 patients were referred from general practitioners and other hospitals; a further 32 patients with a family history of PD were self-referred. This was in response to a local newsletter by the National Norwegian PD Association. Patients with a family history of PD were asked to inform their family members of this research. Any family members who expressed an interest in participating were invited to take part.

A full history, including a family history and neurological examination, was completed for each patient. Clinical criteria for a diagnosis of PD were consistent with possible or probable PD as proposed by Gelb and colleagues (5). Patients demonstrating severe autonomic dysfunction, poor response upon dopaminergic treatment or early dementia were not included. Clinical judgment and the Mini Mental State Examination (MMSE) were used to assess cognitive function. All patients underwent routine laboratory blood testing, and blood samples for DNA extraction and genetic testing were obtained.

Five hundred and nineteen control individuals without signs of a movement disorder were recruited from the same region of Central Norway. Characteristics of patients and controls included in the study are listed in Table 5.

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Table 5. Demographic information on patients with PD included in Paper I, II and V.

Groups	n	Gender (%)	Disease onset (years)	Range (years)	Age at last exam
PD patients	435	174 F (40)	60.3 ± 10.9	33-88	70.2 ± 9.3
		261 M (60)	57.6 ± 10.9	28-80	66.3 ±10.8
Controls	519	233 F (45)	-	47-96	65.8 ±12.2
		286 M (55)	-	46-93	62.7 ±10.4

PD – Mayo Clinic

DNA from patients with PD originating from various sites within the United States and from different European countries has been collected by a number of investigators, and is available at the Mayo Clinic Jacksonville. Some of these samples were used in addition to samples from Trondheim in Paper I.

Dementia – Trondheim

For Paper III we used a series of 242 patients recruited from the geriatric and neurological outpatient clinics at St. Olav's Hospital in Trondheim and from local nursing homes. Medical history, clinical and neurological examination were completed by a neurologist (Sigrid Botne Sando) for all patients. Examination included the use of Mini-Mental State Examination (MMSE), Clock Drawing Test (113), Montgomery and Åsberg Depression Rating Scale (MADRS) (114) and the motor examination part of the Unified Parkinson's Disease Rating Scale (UPDRS III). Available relatives were interviewed about the medical, social and family history, the disease course and completed Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE) (115). Medical records, laboratory blood tests and brain images (CT or MRI) were reviewed.

Guidelines given in the International Classification of Diseases (ICD-10) were applied for diagnosing dementia. Patients diagnosed with AD fulfilled NINCDS-ADRDA criteria for possible or probable AD (15). A diagnosis of probable and possible DLB was made according to the consensus guidelines (116). The

DSM-IV criteria were used for vascular dementia (VaD), and frontotemporal dementia (FTD) was diagnosed according to the Lund-Manchester criteria (117).

The distribution of diagnoses, MMSE scores and demographic data is shown in Table 6. 103 of the patients (43%) were living in nursing homes, and these individuals were examined there. A positive family history of dementia in at least one first-degree relative was noted in 134 (55 %) of the patients. Description of brain imaging (cerebral MRI or CT) was available in 221 (92%) of the patients.

Table 6. Demographic information on patients included in Paper III.

Clinical diagnosis	Number of samples	Gender (%)	Disease onset (years ± SD)	MMSE (mean ± SD)	Years of education (mean ± SD)
AD	161	F 108 (67)	74.2 ± 8.5	14.1 ± 7.8	8.7 ± 2.2
		M 53 (33)	72.9 ± 8.4	16.0 ± 7.5	9.4 ± 2.6
DLB	30	F 13 (43)	71.2 ± 8.6	20.4 ± 5.6	10.2 ± 2.9
		M 17 (57)	71.2 ± 9.1	17.8 ± 8.5	10.0 ± 3.0
FTD	8	F 5 (63)	66.4 ± 13.6	19.8 ± 9.6	12.6 ± 1.9
		M 3 (37)	62.7 ± 12.9	20.0 ± 3.5	9.7 ± 1.5
VaD	43	F 27 (63)	76.1 ± 5.8	18.8 ± 5.7	8.7 ± 2.4
		M 16 (37)	71.4 ± 10.5	17.8 ± 7.6	10.7 ± 3.0
Total number	242	F 153 (63)	74 ± 8.5	15.7 ± 7.7	8.9 ± 2.4
		M 89 (37)	72 ± 9.1	16.8 ± 7.6	9.7 ± 2.7

AD – Alzheimer's disease, DLB – Dementia with Lewy bodies, FTD – Frontotemporal dementia, VaD – Vascular dementia. Three of the patients diagnosed with FTD also had motor neuron disease (FTD-MND), which was confirmed by neurophysiological examinations

3.2 Brain tissue

In Paper IV, we used tissue from several brain banks available at the Department of Neuroscience, Mayo Clinic Jacksonville. Cases with clinical or pathological features consistent with parkinsonism came from the Mayo Clinic Jacksonville brain bank and the University of Miami/National Parkinson Foundation Brain Endowment Bank. The screened samples had received a pathological diagnosis of PD or LBD (n=405), PSP (n=326), and MSA (n=43). Control groups for this study consisted of brains of clinically normal, aged individuals (n=156) and subjects with dementia, most of whom had been referred to the State of Florida Alzheimer's Disease Initiative Brain Bank (AD; n=654).

The study presented in Paper IV was based on archival brains, and therefore details on family history of neurological disease were incomplete and not routinely recorded in the database. Available medical records were reviewed for family history and additional information was obtained from the referring physician. However, the available information was not collected in a standardized manner.

4. Methods

4.1 Molecular biology

Genomic DNA from study individuals and brains were extracted from whole blood and brain tissue using different standard methods. Polymerase chain reaction (PCR) amplifications were performed on thermal cyclers using the specific primers and conditions as described in Paper I-V. After PCR the *LRRK2* and *PINK1* genes were sequenced using the same primers as for the PCR and the BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems). Subsequent capillary electrophoresis was carried out on an ABI 3100 automated capillary machine (Applied Biosystems). Heterozygote base calls and sequence alignment were performed with Sequencher (Gene Codes Corp.).

In addition to direct sequencing, mutation screening was performed using several different methods. Some missense mutations result in the loss of a recognition sequence for a particular restriction enzyme. This enzyme can be used to genotype the sample without completely sequencing it, by analyzing restriction fragment length polymorphisms on an agarose gel after PCR and subsequent digestion with the enzyme. The Lrrk2 R1441C/H/G substitutions were genotyped using the Bst*U*I enzyme (Figure 5).

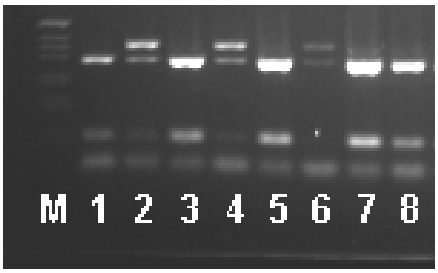

Figure 5. An agarose gel image of the *Bst*UI digestion for the identification of the mutant allele. Lane M contains a 1Kb DNA size ladder. Lanes 1, 3, & 5 are DNA samples and lanes 2, 4 & 6 are positive controls for the heterozygous R1441C/H/G, respectively.

DNA was genotyped for several of the other mutations using allelic discrimination assays, employing TaqMan chemistry on an ABI 7900 (Applied Biosystems). Analyses were performed using Sequence Detection System 2.2 software (Applied Biosystems).

In the study presented in Paper I we genotyped 17 microsatellite markers for linkage and haplotype analyses. Seven published microsatellite markers were chosen to span the *PARK8* region (D12S87, D12S1648, D12S2080, D12S2194, D12S1048, D12S1301 and D12S1701). *LRRK2* is located between D12S2194 and D12S1048. In addition to these seven, we developed ten novel microsatellite markers in this region by searching for repeat polymorphisms using RepeatMasker of *in silico* BAC sequence (UCSC Human Genome Browser Web site).

For the genotyping of microsatellite markers one primer of each pair was labeled with a fluorescent tag. PCR reactions were carried out under standard conditions and PCR products were run on an ABI 3100 genetic analyzer. Results were analyzed using Genescan 3.7 and Genotyper 3.7 software (Applied Biosystems). Marker allele frequencies were not publicly available for the novel markers and they were therefore estimated by genotyping 93 unrelated North American individuals.

In Paper V we designed an assay to detect multiplications and deletions of the *PINK1* gene. Absolute quantitative PCRs of *PINK1* were performed using the iQ SYBR Green Supermix kit (Biorad). Absolute quantification of DNA template was calculated from a standard curve using the MJ Opticom Monitor v.3.1. For this assay the concentrations of *PINK1* exon 4 and 7 were individually analysed and compared with concentrations of the external control gene, human serum albumin. Each sample was run in a triplicate reaction.

Relative gene dosage ratios with standard deviations were calculated by dividing the normalised mean *PINK1* quantity by the mean albumin quantity. The advantage of this assay was that we could design positive controls for deletion and multiplication mutations by using other amounts of DNA (Figure 6). A relative ratio with standard deviations between 0.75 and 1.25 was considered normal, a heterozygous deletion was expected at a ratio between 0.25 and 0.75, and a duplication was expected between 1.25 and 1.75. Ambiguous samples were re-run in triplicate with DNA from a separate tube.

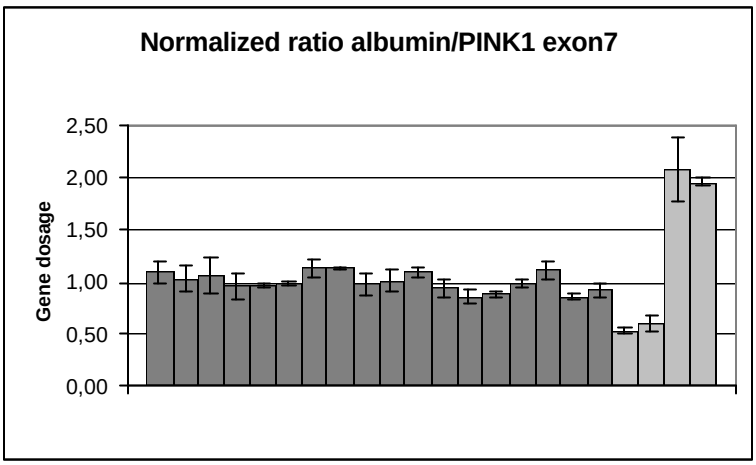

Figure 6. Relative ratios of gene dosage of *PINK1* compared to albumin for 17 EOF patients (dark grey); the bars represent standard deviations. The ratios for two deletion controls and two triplication controls are shown in light grey.

4.2 Pathology

In Paper IV neuropathological review of available autopsy material from subjects carrying the G2019S mutation performed. Postmortem examination of the brain followed standard protocols to identify macroscopic and microscopic evidence of disease. Sections from tissue blocks were stained with hematoxylin and eosin, thioflavin-S and anti-a-synuclein antibodies. A pathological diagnosis of LBD had been made based on the presence of classic intracytoplasmic Lewy bodies within neurons of pigmented brain stem nuclei and/or similar inclusions in limbic and neocortical regions.

For quantitative assessment areas chosen to represent brain stem, paralimbic areas and neocortex were studied. Cortical Lewy bodies were quantified using a-synuclein immunohistochemistry in four cortical regions at magnification x200. In each case Lewy body distribution and frequency was evaluated using the consortium guidelines Lewy body scores, and the case were classified into brainstem dominant, transitional and diffuse categories (116).

Alzheimer's disease pathology was assessed by the use of thioflavin-S fluorescence microscopy in four cortical regions. Average density of senile plaques at magnification x100 and of neurofibrillary tangles (NFT) at

magnification x400 was calculated. Braak NFT staging was performed (118). The NFT stages range from 0 to VI, with IV and greater characteristic of AD.

4.3 Statistics

Linkage

Linkage is the tendency for genetic markers to be inherited together in case of recombination of the genetic material because of their location near one another on the same chromosome. It is possible to calculate the overall likelihood of a pedigree, on the alternative assumptions that the loci are linked (recombination fraction = ?) or not linked (recombination fraction = 0.5). The ratio of these two likelihoods gives the odds of linkage, and the logarithm of the odds is the LOD score. Morton demonstrated that LOD scores represent the most efficient statistic for evaluating pedigrees for linkage (119). In a set of families, the overall probability of linkage is the product of probabilities in each individual family, therefore LOD scores can be added up across families.

Linkage analysis can be more efficient if data for more than two loci are analyzed simultaneously, as this overcomes problems caused by limited informativity of markers. In Paper I, we calculated multipoint LOD scores for all families under the assumption of an autosomal dominant model by using the program GENEHUNTER-PLUS (120). The frequency of the deleterious allele was set at 0.0001; marker allele frequencies were determined empirically. The map positions for each marker were taken from Rutgers combined linkage-physical map version 1.0 (MAP-O-MAT web site). For tightly linked loci with no observed recombinants, inter-marker genetic distances were assigned as 0.01cM.

Association

Genetic association is the occurrence together in a population, more often than can be readily explained by chance, of two or more traits of which at least one is known to be genetic. Association between genetic polymorphisms and disease was in Paper V calculated by using Pearson's chi-square test.

Penetrance

Age-dependent penetrance was in Paper I estimated as the probability of a gene mutation carrier becoming affected, at a given age, within the families. The number of affected mutation carriers within 5-year age groups was divided by the total number of carriers (both affected and unaffected) within that group.

Haplotype analysis

A haplotype is a particular combination of alleles that are closely linked on a chromosome. In Paper I, haplotypes were established manually for families with known phase, after genotyping 21 polymorphic genetic markers (17 microsatellites and 4 SNPs). Haplotype frequencies in the general population were estimated from genotypes of 93 unrelated individuals by use of an estimation-maximization algorithm (121).

4.4 Ethics

All patients and controls included in the studies have provided informed consent. Study protocols for both the sample series from Trondheim (Parkinson's disease and dementia) have been approved by the Regional Committee for Medical Research Ethics in Central Norway. Studies performed at the Mayo Clinic Jacksonville have been approved by the Mayo Clinic Institutional Review Board. The biobanks in Trondheim have the necessary approval required by Norwegian biobank law.

Results of genetic examinations as a part of the studies have not been given to patients and family members participating in the study. The identity of study participants has remained unknown for researchers working on the projects, except for those who have been involved in clinical examinations of patients.

5. Results

5.1 Review of paper I

Identification of a novel *LRRK2* mutation linked to autosomal dominant parkinsonism: Evidence for a common founder across European populations.

Jennifer Kachergus*, Ignacio F. Mata*, Mary Hulihan, Julie P. Taylor, Sarah Lincoln, Jan Aasly, J. Mark Gibson, Owen A. Ross, Timothy Lynch, Joseph Wiley, Haydeh Payami, John Nutt, Demetrius M. Maraganore, Krzysztof Czyzewski, Maria Styczynska, Zbigniew K. Wszolek, Matthew J. Farrer, and Mathias Toft *Both authors contributed equally

Background: Autosomal dominant parkinsonism has been attributed to pathogenic amino acid substitutions in Leucine-rich repeat kinase 2 (Lrrk2).

Methods and results: By sequencing multiplex families consistent with a *PARK8* assignment, we identified a novel heterozygous *LRRK2* mutation. A referral sample of 248 affected probands from families with autosomal dominant parkinsonism was subsequently assessed; 7 (2.8%) were found to carry a heterozygous *LRRK2* c.6055G>A transition (G2019S). These seven patients originate from the United States, Norway, Ireland, and Poland. In samples of patients with idiopathic Parkinson disease (PD) from the same populations, further screening identified six more patients with Lrrk2 G2019S; no mutations were found in matched control individuals. Subsequently, 42 family members of the 13 probands were examined; 22 have an Lrrk2 G2019S substitution, 7 with a diagnosis of PD. All patients share an ancestral haplotype indicative of a common founder. Within families, Lrrk2 G2019S segregates with disease (multipoint LOD score 2.41). Penetrance is age dependent, increasing from 17% at age 50 years to 85% at age 70 years.

Conclusion: Our study demonstrates that Lrrk2 G2019S accounts for parkinsonism in several families within Europe and North America. Our work highlights the fact that a proportion of clinically typical, late-onset PD cases have a genetic basis.

5.2 Review of paper II

Clinical features of *LRRK2*-associated Parkinson's disease in Central Norway

Jan O. Aasly, Mathias Toft, Ignacio F. Mata, Jennifer Kachergus, Mary Hulihan, Linda R. White, and Matthew Farrer.

Background: Several pathogenic mutations in the *leucine-rich repeat kinase 2* (*LRRK2*; PARK8) gene have recently been identified in familial and sporadic parkinsonism. Our objective was to present a detailed clinical study of Norwegian patients with *LRRK*-associated disease.

Methods: We screened 435 Norwegian patients diagnosed with Parkinson's disease and 519 control subjects for the presence of 7 *LRRK2* mutations previously published. Patients were clinically examined and followed longitudinally.

Results: Nine patients from seven families were found to be heterozygote carriers of the *LRRK2* c.6055G>A (G2019S) mutation. Twelve of 28 first-degree relatives also carried the mutation, but only 1 had Parkinson's disease. The clinical features included asymmetric resting tremor, bradykinesia, and rigidity with a good response to levodopa and could not be distinguished from idiopathic Parkinson's disease. No patient carried any of the other *LRRK2* mutations.

Conclusions: Patients with a Lrrk2 G2019S substitution present with a levodopa–responsive parkinsonian syndrome with asymmetric resting tremor, bradykinesia, and rigidity, which are typical of idiopathic PD. The nonmotor autonomic, psychiatric, and cognitive symptoms are mild. Currently, Lrrk2 G2019S–associated parkinsonism is the most prevalent cause of genetically determined PD in Norway.

5.3 Review of paper III

LRRK2 mutations are not common in Alzheimer's disease

Mathias Toft, Sigrid Botne Sando, Stacey Melquist, Owen A. Ross, Linda R. White, Jan O. Aasly, Matthew J. Farrer

Background: The development of common age-related neurodegenerative disorders as Parkinson's disease and Alzheimer's disease (AD) is influenced by genetic factors. Recently, pathogenic mutations in the *leucine-rich repeat kinase 2 (LRRK2)* gene have been identified in familial parkinsonism. Individuals in some of these families developed symptoms of dementia with Lewy-bodies and AD. The *LRRK2* gene is also located within a locus on chromosome 12 reported in late-onset AD, and is therefore a good candidate gene for dementia.

Methods: A series of 242 patients from Norway diagnosed clinically with dementia were included in the study, the majority being diagnosed with AD (n=161). 43 patients with vascular dementia, 30 patients with dementia with Lewy bodies and 8 with frontotemporal dementia were also included. Individuals were screened for the presence of seven known pathogenic mutations previously reported in the *LRRK2* gene.

Results: We did not identify *LRRK2* mutations in our series of dementia patients.

Conclusion: Our results indicate that known pathogenic mutations are not common in patients clinically diagnosed with AD. However, these results do not exclude a possible role of other genetic variants within the *LRRK2* gene in AD or other forms of dementia.

5.4 Review of paper IV

Lrrk2 and Lewy Body Disease

Owen A. Ross, Mathias Toft, Andrew J. Whittle, Joseph L. Johnson, Spiridon Papapetropoulos, Deborah C. Mash, Irene Litvan, Mark F. Gordon, Zbigniew K. Wszolek, Matthew J. Farrer, and Dennis W. Dickson

Background: The Lrrk2 kinase domain G2019S substitution is the most common genetic basis of parkinsonism. Patients harboring the G2019S substitution usually present with clinical PD, but pathology has yet to be clearly defined. The rationale for our study was to provide a consensus on the pathology associated with Lrrk2 G2019S–associated disease.

Methods: We screened the Mayo Clinic Jacksonville brain bank and University of Miami/National Parkinson Foundation Brain Endowment Bank for cases with clinical or pathological features consistent with parkinsonism. The screened samples included PD or LBD (n=405), PSP (n=326), and MSA (n=43). Control groups consisted of brains of clinically normal, aged individuals (n=156) and subjects with AD (n=654). DNA was obtained from frozen brain tissue and genotyped for the exon 41 *LRRK2* 6055G>A (G2019S) mutation. Available medical records from mutation carriers were reviewed and neuropathological examination was performed.

Results: Lrrk2 G2019S was observed in approximately 2% (n = 8) of our PD/LBD cases (n = 405). The mutation was also found in one control and one Alzheimer's disease patient, reflecting reduced penetrance. Neuropathological examination showed typical Lewy body disease, with brainstem-type LBD (n=4), transitional LBD (n=3), and diffuse LBD (n=1).

Conclusion: The most common neuropathology of G2019S-associated PD is Lewy body disease. Therapeutic strategies targeted at modulating Lrrk2 kinase activity may be important to treat patients with genetically defined familial or typical sporadic PD.

5.5 Review of paper V

***PINK1* mutation heterozygosity and the risk for Parkinson's disease**

Mathias Toft, Ronny Myhre, Liza Pielsticker, Linda R. White, Jan O. Aasly, and Matthew J. Farrer

Background: Mutations in the *PINK1* gene have been identified in recessively inherited and seemingly sporadic early-onset parkinsonism (EOP).

Objective: Our objective was to further evaluate the pathogenic role of *PINK1* mutations in familial and sporadic PD.

Methods: We included a total of 131 patients diagnosed with PD from an ongoing study of the genetics of PD in Norway. Eighty-nine subjects had EOP (onset =50 years); the remaining had familial late-onset disease (mean age at onset 64 years). *PINK1* analysis included complete sequencing and an assessment of gene dose alterations. Mutations identified were examined in 350 ethnically matched control individuals.

Results: Heterozygous missense mutations in *PINK1* were found in three of 131 patients; none of the patients carried homozygous or compound heterozygous mutations. One of these three patients had a father affected by PD, and he carried the mutation. In addition, three novel and seven known polymorphic variants were identified in the gene, although none appeared associated with disease risk. A parkinsonian phenotype, with asymmetric onset and without any atypical features, characterised the clinical presentation of the three patients with heterozygous mutations.

Conclusions: Mutations in the *PINK1* gene are rare in Norwegian early-onset and familial PD patients. However, our data suggest that some heterozygous mutations might increase the risk of developing PD.

6. Discussion

6.1 Identification and evidence for pathogenicity of Lrrk2 G2019S

In Paper I we identified a novel c.6055G>A mutation in the *LRRK2* gene. This mutation was found by sequencing of genomic DNA from probands of six small families with positive LOD scores for markers within the PARK8 locus from a previous study (108). Affected members of one of the six families, Family 3211 originating from Ireland, carried this mutation. No *LRRK2* mutation was found in the remaining five families.

We screened for this specific mutation in an additional 242 affected probands of familial parkinsonism consistent with autosomal dominant parkinsonism. In addition, we examined a total of 806 patients with sporadic PD from Norway, Ireland and Poland, and 2260 controls. The results of this screening and subsequent analyses provided strong evidence for pathogenicity of the identified mutation.

Genetic evidence

The c.6055G>A mutation causes an amino acid alteration in the predicted Lrrk2 protein, replacing a glycine residue with a serine at position 2019 (p.Gly2019Ser = G2019S). This amino acid is located within a region evolutionary highly conserved across species (Figure 7). In addition, a previously described I2020T mutation affects an adjacent codon, further highlighting the functional importance of the region.

However, not only is this residue conserved across species, but also between different human kinases. The substitution is localized within the functionally important kinase domain of the Lrrk2 protein, which belongs to the MAPKKK subfamily of kinases. The active site of kinases is located in a cleft between an N-terminal and a C-terminal lobe and is covered by an activation segment in its inactive form. The activation segment is a region of the kinase domain that undergoes crucial structural changes necessary to allow access to peptide

substrates and also to orientate key catalytic amino acids within the cleft of the kinase (122). In different kinases, the activation segment starts and ends with the conserved residues DF/YG and APE, respectively (123). The Lrrk2 G2019S substitution changes the highly conserved glycine (G) at the start of this segment (Figure 8).

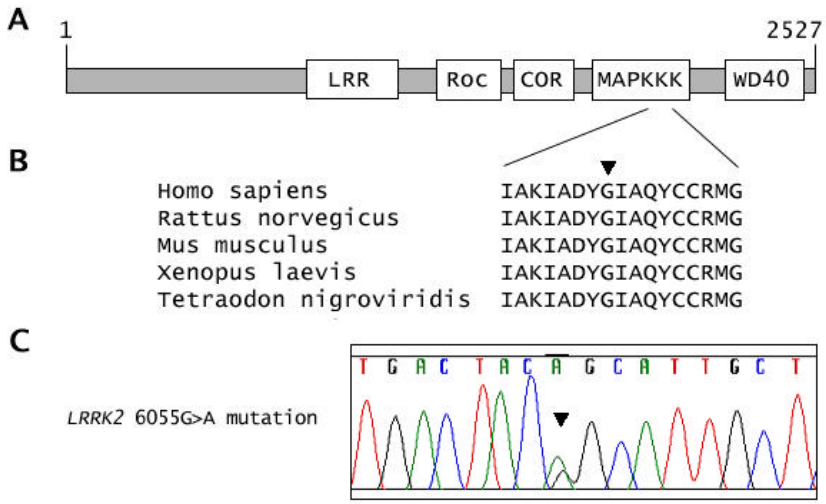

Figure 7. Lrrk2 with the novel G2019S substitution

a) Schematic drawing of Lrrk2 with predicted protein domains

b) The human Lrrk2 protein sequence in the region of the G2019S mutation aligned with orthologs from rat, mouse, frog and puffer fish.

c) Chromatogram showing the c.6055G>A mutation (G2019S).

In other kinases, oncogenic mutations in residues within the activation segment of the kinase domain have an activating effect (124). We therefore postulated in Paper I, and in an additional letter in the Lancet, that mutations in this region might have an activating effect on the kinase activity of Lrrk2 (125). A mutation causing “gain of function” of the resulting protein would also be compatible with the dominant mode of disease transmission observed in the families. The two pathogenic mutations identified in this region introduce serine (G2019S) and threonine (I2020T) residues, which may be potential targets for phosphorylation, increasing activity or altering substrate specificity.

```
LRRK2      DYGIAQ----YCCRMGIKTSEGTPGFRAPE
LRRK1      DYGISR----QSFHEGALGVEGTPGYQAPE
MATK       DFGLAK-----AERKGLDSSRLPVKWTAPE
PDGFRA     DFGLARDIMHDSNYVSKGSTFLPVKWMAPE
MAP3K10    DFGLAR----EWHKTTKMSAAGTYAWMAPE
DAPK1      DFGN-----------EFKNIFGTPEFVAPE
BRAF       DFGLATVKSRWSGSHQFEQLSGSILWMAPE
```

Figure 8. Aligned amino acid sequences of the activation segment of human kinases.

Statistical evidence

In Paper I the Lrrk2 G2019S mutation co-segregated with disease within all families where the mutation was found in more than one affected individual. However, one affected member of one family did not carry the disease-associated *PARK8* haplotype. He had akinetic rigid parkinsonism unresponsive to levodopa, and he was thus considered a phenocopy and excluded from further analyses.

Evidence for linkage to the *PARK8* locus was found across families, with a combined maximum multipoint LOD score of 2.41, corresponding to a *P* value of 4.3×10^{-4}. Positive LOD scores were found in all families. As only a defined chromosomal region was investigated, rather than a genome-wide search, the mLOD score exceeds that required for significance, P=0.01 (126).

In total, we found the G2019S mutation in seven of 248 families with autosomal dominant parkinsonism (2.8%) and six of 806 patients with seemingly sporadic PD (0.7%). We did not identify the mutation in any of 2260 control individuals, demonstrating a clear association between the variant and disease. At the same time as Paper I was published, three other groups reported the finding of G2019S in a number of patients from several populations. This established that the mutation is relatively frequent in PD and rare in controls (127-129). Numerous studies from a range of different populations have now confirmed these findings.

Evidence from functional studies

Several studies have indicated that the G2019S mutation increase the kinase activity of the protein, as proposed in Paper I. West and colleagues found that the Lrrk protein is mainly localized in cytosplasm, but also associates with the mitochondrial outer membrane. In an *in vitro* kinase assay, the G2019S mutation caused an increase in autophosphorylation and phosphorylation of myelin basic protein (130). Mutant Lrrk2 causes degeneration in different cell lines, including neuronal cells (131). This cell death is dependent on the kinase activity, which is regulated by GTP via the Lrrk2 Roc domain (132, 133). The substrates of Lrrk2 and mechanisms of neuronal cell death are still unknown.

6.2 Frequency of LRRK2 mutations

In Paper I we identified the G2019S mutation in seven of 248 families with autosomal dominant parkinsonism (2.8%) and six of 806 patients with seemingly sporadic PD (0.7%). Screening of 6 other mutations in the Norwegian patients included in Paper I did not reveal any other *LRRK2* mutations in our population (Paper II). In Paper IV we found G2019S in 8/405 pathological cases with LBD (2%). Our conclusion is that, at least in Norway, G2019S is the most frequent pathogenic Lrrk2 amino acid substitution and the most common known genetic cause of PD. On the other hand, other *LRRK2* mutations are relatively rare in Norway.

The frequency of G2019S varies between different studies. In studies of this mutation in familial or autosomal dominant PD, the frequency has varied between 0% and 37% (134, 135). Each study used different criteria for autosomal dominant or familial parkinsonism, which probably explains some of the different mutation frequencies identified. In Paper I, we used a relatively liberal criterion for inheritance compatible with autosomal dominant disease, whereby at least two affected individuals in two consecutive generations were required for inclusion into the study.

The frequency of G2019S also seems to be population specific. The highest mutation frequencies have been found among North African Arabs (41%) and Ashkenazi Jews (18%), also suggesting the likely region of origin of the

mutation (135, 136). It is likely that the mutation has been spread by migration of Arab and Jewish populations, indicated by a South to North gradient of mutation frequency. The mutation frequency is high in Spain and Portugal, where G2019S has been found in between 3% and 8% of studied patients (137-139). In Northern European and North American populations, which mainly is of Northern European origin, most studies have found a mutation frequency of G2019S between 0.5% and 1.5% (128, 140-142), comparable to our results from the Norwegian, Irish and Polish populations. In Asian populations G2019S seems to be very rare (143-145).

The frequencies reported might be overestimates of the true prevalence in the different populations. Most studies have been performed in clinic based series, mainly from movement disorders specialists, including the series we used in Papers I and II. This might bias the results, as patients with familial disease could be more aware of the disease and seek specialized hospital care (referral bias). The mutation prevalence in a community-based PD cohort from the UK was 0.4% (146), and a similar study from the US showed a prevalence of 0.5%. Both these studies found lower mutation frequencies than studies from the same countries using clinic-based study designs.

Several other mutations than G2019S have been identified in the *LRRK2* gene, both in families with autosomal dominant parkinsonism and in individuals with seemingly sporadic disease. Segregation analyses within families provides statistical evidence for the pathogenicity of some of the published mutations (R1441C, R1441G, Y1699C, G2019S and I2020T).

A number of additional mutations have been published (Table 7). However, these variants have been identified only in small families or single individuals. In a paper not included in this thesis we have examined the role of one of these variants in PD (R1514Q), and demonstrated that this variant is not associated with disease and does not segregate within a large family (147). Similar studies are needed for each variant to determine its pathogenicity. They should therefore be considered putative pathogenic variants until more data is available and are not discussed further in this thesis.

Table 7. Putatively pathogenic LRRK2 mutations

Exon	Amino Acid Change	Protein Domain	Reference
19	R793M		Berg et al 2005
21	Q930R		Berg et al 2005
23	R1067Q	LRR	Skipper et al 2005
24	S1096C	LRR	Berg et al 2005
24	L1114L	LRR	Zimprich et al 2004
25	I1122V	LRR	Zimprich et al 2004
25	A1151T	LRR	Schlitter et al 2006
27	S1228T	LRR	Berg et al 2005
29	I1371V	Roc	Paisan-Ruiz et al 2005
31	R1441H	Roc	Mata et al 2005
	IVS31+3 A>G	Roc	Zabetian et al 2005
32	R1514Q	COR	Mata et al 2005
	IVS33+6 T>A	COR	Skipper et al 2006
38	M1869T	COR	Mata et al 2005
38	G1874X	COR	DiFonzo et al 2005
39	R1941H	MAPKKK	Khan et al 2005
41	I2012T	MAPKKK	Tomiyama et al 2006
47	T2356I	WD40	Khan et al 2005
48	G2385R	WD40	Mata et al 2005

To date, few studies have reported the frequency of *LRRK2* mutations other than G2019S. The R1441G mutation was common (8%) in a series of patients from the Basque population (Paisan-Ruiz *et al*. 2004), and this mutation has also been found in patients from other regions of Spain (Mata *et al*. 2005b). R1441G seems far less frequent in other populations. The R1441C mutation has been identified in patients from different populations, indicating that this mutation might be the second most common outside Spain (111, 148, 149).

6.3 Penetrance and haplotype analyses

Penetrance estimations

As with PD in general, age is a risk factor for *LRRK2*-associated parkinsonism. The age of onset is variable, ranging from the fourth to the ninth decade, with the average age of onset between 55 and 65 years in the various families and

studies (109, 111, 129). Penetrance of *LRRK2* mutations depends on age and estimates vary among mutations and populations.

In Paper I we calculated penetrance of the G2019S mutation and found that it increases in a close to linear fashion from 17% at age 50 years to 85% by age 70 years (Figure 9). Age at onset was variable, both within and between different families, with a mean age at onset of 56.8 years. Most mutation carriers had late-onset disease (>50 years of age). The variable age at onset suggests that other susceptibility factors, environmental or genetic, might influence the phenotype. Since the penetrance of *LRRK2* mutations depends on age, mutations are also found in patients with a negative family history for PD and seemingly sporadic disease. This has important implications for genetic screening and counseling of PD patients.

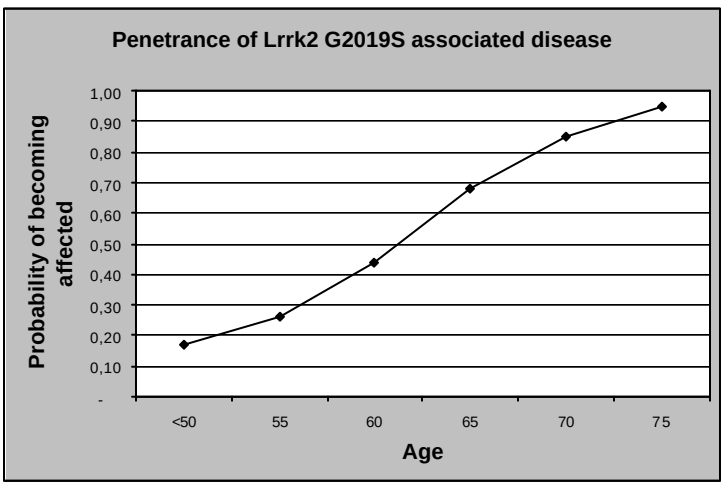

Figure 9. Probability of becoming affected by parkinsonism, in Lrrk2 G2019S carriers, as a function of age.

Lifetime penetrance of the G2019S mutation has also been calculated for the Ashkenazi Jewish population. A penetrance of around 35% was found using different methods than we used in Paper I (136). Our results could be an overestimate, since some of the patients in our study were from selected families. Penetrance in families with a clear autosomal inheritance is likely to be higher than in the general population. Despite this, a penetrance of 35% seems low, taking into account the low frequency of G2019S found in control

individuals. Ideally, penetrance should be calculated from population-based studies, but data from such studies have so far not been published.

Few penetrance estimates have been published for other mutations than G2019S. In the Sagamihara kindred, segregation analysis of the disease-associated haplotype indicated penetrance of about 65% (106). The oldest age at onset in families with the R1441G substitution was 80 years (110), indicating a high penetrance in this age group.

Haplotype analyses

All mutation carriers in Paper I, although a family history was not always apparent, shared a small ancestral haplotype of a size of 145 to 154 kb. This indicated that the mutation arose in an ancient common founder. It also suggested that this specific mutation is frequent and spread throughout variuos populations, as now demonstrated by the identification of G2019S in a high number of populations.

Other studies have also demonstrated that most European and North American G2019S carriers share a haplotype (150, 151). Lesage and colleagues found that the region shared was only 60 kb (151). However, it has been shown that one of the markers (D12S2515) used in both this and our study is unstable. Thus, allelic differences observed among haplotypes at this marker could be due to recurrent mutations rather than recombination, affecting the calculated size of the shared chromosomal region (145). Separate founding events for G2019S have recently been suggested for a proportion of North American and Japanese patients with this mutation (145, 152).

6.4 Clinical features of LRRK2-associated parkinsonism

In Paper II we studied in detail the clinical presentation of G2019S-associated PD in 10 Norwegian patients. Our main conclusion was that motor symptoms, cognitive performance and response to dopaminergic treatment were indistinguishable from those observed in patients with sporadic late-onset PD.

In our study, resting tremor was the presenting symptom in six of ten patients. All except one patient subsequently developed resting tremor during the course of their disease. Bradykinesia was a consistent finding in all patients and was the first symptom observed in three of them. The poverty of movement was generally moderate and episodes of severe akinesia were only observed in off-periods in two patients.

Nine of the 10 patients had a good to excellent response to levodopa or other dopaminergic agents. In one patient the response was less profound. Levodopa-induced dyskinesias were observed in six patients. The dyskinesias were most pronounced in the two early-onset cases. Good response to dopaminergic treatment and frequent development of dyskinesias are also found by other investigators (129, 150). One study found less severe clinical symptoms in G2019S carriers, despite an increased disease duration, compared with other patients with familial parkinsonism (129).

We found no indication of early dementia, autonomic dysfunction, or other atypical neurologic signs being more frequent than in other patients with PD, which is in accordance with other studies (127, 150). Of interest, levodopa-responsive foot dystonia has been the initial symptom in several patients, including one patient in Paper I (128, 150). Further studies are needed to assess the frequency of dystonia, but dystonia might be more frequent in *LRRK2*-associated parkinsonism.

The results in Paper II are similar to the findings in patients with other pathogenic *LRRK2* mutations (Table 8). In the Sagamihara kindred (I2020T), affected members had asymmetric parkinsonism with a favorable response to dopaminergic treatment, and none of the individuals presented with any atypical symptoms (106, 107). Symptoms typical of sporadic PD responding to levodopa treatment were also found in the German family with the I2020T mutation (111).

Slowly progressive parkinsonism with tremor as the presenting and initially predominant symptom was reported in R1441G-associated disease (109). These patients had a good response to dopaminergic treatment and developed

motor complications typical of PD after 6 to 8 years of treatment. No cognitive decline was observed even after long disease duration, but delusional and paranoid hallucinations occurred in one affected individual. Writer's cramp and foot dystonia were present in a member of one family (109). In a second report, the clinical features were consistent with sporadic late-onset PD (153).

Resting tremor was not as prominent in Family D (Western Nebraska), a large family with an R1441C substitution affecting the same codon as the variant found in the Basque and Spanish families. The most common initial presentation was bradykinesia (60%) and unilateral resting hand tremor (40%). Response to levodopa therapy was excellent, and motor complications have developed in half the patients receiving treatment. The phenotype of this family is indistinguishable from typical late-onset PD, except in one family member who also developed supranuclear gaze palsy but remained responsive to levodopa until death (154, 155).

Other atypical symptoms have been observed in a second family, Family A (German-Canadian), carrying the Y1699C substitution. This family is characterized by a parkinsonian syndrome responsive to dopaminergic treatment, with subsequent development of motor complications. However, one family member had clinical amyotrophy characterized by muscle weakness, atrophy, and fasciculations. Two other mutation carriers in this family presented with dementia (111, 156).

The number of patients studied so far is limited, especially for other mutations than G20192S. Further studies are needed to determine if the atypical symptoms found in some families with *LRRK2* mutations are typical for *LRRK2*-associated PD. The main findings from clinical studies support our results in Paper II. The clinical presentation of the disease is heterogeneous, but the vast majority of patients have clinical symptoms within the spectrum normally found in idiopathic PD, and they fulfill diagnostic criteria for the disease.

Table 8. Comparison of clinical features of pathogenic mutations within the *LRRK2* Gene[+]

	Roc		COR	MAPKKK	
Domain and mutation (exon)	R1441C (31)	R1441G (31)	Y1699C (35)	G2019S (41)	I2020T (41)
Family, no.	2	4	1	7	1
Affected individuals, no.	29	35	15	10	7
Mean (range) age at onset, y	63 (48-78)	61 (50-79)	53 (36-65)	57 (43-70)	54 (48-59)
Mean (range) disease duration, y	14 (4-26)	NA	13 (5-18)	14 (5-25)	20 (12-27)
Predominant initial sign	RT/B	RT	RT	RT/B	B
Parkinsonism					
Resting tremor	+	+	+	+	+
Bradykinesia	+	+	+	+	+
Rigidity	+	+	+	+	+
Postural or gait instability	+	+	+	+	+
Asymmetry of parkinsonism	+	+	+	+	NA
Response to levodopa	+	+	+	+	+
Levodopa-induced dyskinesia	+	+	+	+	–
Clinical phenotype	PD	PD	PD-plus syndrome	PD	PD
Other clinical features	-*	Dystonia, delirium	Dementia, amyotrophy, dystonia	Dystonia, dementia, depression, RLS	-

B, Bradykinesia; COR, domain C-terminal of Roc; MAPKKK, mitogen-activated protein kinase kinase kinase; NA, not available; PD, Parkinson's disease; Roc, Ras in complex protein; RT, resting tremor.

[+]Data from Zimprich *et al*. 2004; Aasly *et al*. 2005; Paisan-Ruiz *et al*. 2005.

[*]One case with supranuclear palsy responsive to levodopa therapy.

6.5 Neuropathology of LRRK2-associated parkinsonism

In Paper IV we screened DNA from a large number of cases (total n=1584) to establish the neuropathology associated with the Lrrk2 G2019S mutation. The DNA was obtained from several brain banks and included cases with neuropathologies typical of different synucleinopathies and tauopathies, as well as normally aged individuals. We identified a total of 10 cases with the mutation; eight with Lewy body disease (LBD), one with Alzheimer pathology (discussed below) and one normally aged brain without any significant neuropathology.

The main conclusion of Paper IV is that the most common neuropathology of G2019S-associated Parkinson's disease is Lewy body disease. In all respects, the LBD was typical in these cases, with prominent involvement in the brainstem monoaminergic nuclei, basal forebrain, and limbic cortex. Neuropathologic examination revealed brainstem-type LBD in 4 caes, transitional LBD in 3, and diffuse LBD in 1 case. Only three of the cases had a known family history of PD, confirming the occurrence of this mutation in both familial and sporadic PD.

Four of the cases had autonomic dysfunction. In one case material was available to demonstrate a correlation between these symptoms and the presence of Lewy bodies and Lewy neurites in the autonomic nervous system, including the intermediolateral column of the spinal cord and the myenteric plexus in the large intestine. This demonstrates that, at least in advanced stages, the a-synuclein associated pathology extends beyond the central nervous system.

Braak and colleagues have proposed a neuropathological staging protocol for PD, where the disease process begins in the dorsal nucleus of the vagal nerve. From there, the pathology spreads in an upward path through the lower brainstem and basal forebrain until it reaches the cerebral cortex (9). In our study, we found no apparent relationship between age at disease onset, disease duration, and the stage of LBD observed. The mutation carrier with the longest duration of disease (31 years) had only brainstem LBD. In a study of the association of cognitive status with neuropathologic stages of PD, cognitive

performance measured by the MMSE test did not correlate significantly with disease duration, age at disease onset, or age at death (10). This indicates that, although the pathology might spread in the proposed pattern, the rate of progression of both neuropathological findings and cognitive decline is very different between affected individuals.

Two of the LBD cases in Paper IV presented clinically with dementia. Both had transitional LBD and one of them had sufficient neurofibrillary pathology to warrant a diagnosis of AD. Dementia was not noted in the patient with diffuse LBD, but the clinical information available was incomplete. The main substrate of dementia in PD is LBD (157). Cognitive status of patients and the stage of pathology correlates, indicating that the risk of developing dementia in PD becomes greater as the disease process in the brain progresses (10). However, as also demonstrated in our patients, cognitive decline can develop in the presence of mild PD-related cortical pathology and, conversely, widespread cortical lesions do not necessarily lead to cognitive decline (10, 158). If Lewy bodies do not accurately predict cognitive decline in PD, this raises the question whether Lewy bodies are markers for dying neurons, or if they represent a protective mechanism of surviving cells. Recent studies of other neurodegenerative disorders with inclusion bodies have supported the latter theory. For instance, inclusion body formation reduced the risk of neuronal death in a study of a model of Huntington's disease (159).

The healthy control subject in our study who carried Lrrk2 G2019S died at 68 years of age after an acute myocardial infarction. No significant neurodegenerative pathology was observed. This subject had no documented family history of PD. These findings are consistent with the reduced penetrance of Lrrk2 G2019S already discussed.

Two other reports of autopsy findings for the Lrrk2 G2019S mutation have been published, and support the conclusion that the main pathological substrate is LBD. Histopathologic examination of three patients in a study by Gilks and co-workers showed nigral cell loss with typical Lewy bodies. In two of the three patients, Lewy bodies were present in the limbic cortices, whereas the last case

showed signs of pathologic aging, with diffuse senile plaques and occasional neurofibrillary tangles (128). Giasson and colleagues reported pathological findings in three patients. Two patients demonstrated classical LBD, one patient also had concurrent AD pathology. The third patient was characterized by parkinsonism without Lewy bodies but demonstrated dystrophic neurites in the *substantia nigra* which stained for Lrrk2 using a Lrrk2 antibody (160).

Neuropathologic findings have also been reported for 3 other *LRRK2* mutations. In contrast to the pathology of idiopathic PD, these examinations have shown strikingly diverse and pleomorphic findings. Neuronal loss and gliosis in the *substantia nigra* are found in all cases. However, various intracellular inclusions have been demonstrated.

In the Sagamihara kindred (I2020T), brain autopsy has been reported in 4 cases, which were all diagnosed as “pure nigral degeneration”. The examinations showed mild to moderate nigral degeneration without any Lewy bodies or other coexisting pathology. No other pathologic changes were reported in other nuclei of the basal ganglia, in the cerebral cortex, or in the cerebellum (106, 107).

Four members of Family D (Western Nebraska; R1441C) have come to autopsy, all presenting with neuronal loss and gliosis of the *substantia nigra*. However, variable α-synuclein and tau pathology was demonstrated in the affected individuals, all carrying the same *LRRK2* mutation. Lewy body pathology was found in two cases; in one case, Lewy bodies were restricted to brainstem nuclei, whereas the pathology was more widespread in the second patient. One family member had a tauopathy with neurofibrillary tangles and neuropil threads, qualitatively similar to the pathologic findings characteristic of PSP. The fourth family member showed ubiquitin-immunoreactive neuronal inclusions, without Lewy bodies or tau-related pathology (111, 155).

A number of cytoplasmic and nuclear inclusions were found in two members of Family A (German-Canadian; Y1699C). The nuclear inclusions were similar to

Marinesco bodies, although the neuronal cytoplasmic inclusions appear to be novel and are unclassified. Other patients from Family A had pathologic evidence of minimal anterior horn cell degeneration, indicating mild motor neuron disease, and one individual met neuropathologic criteria for AD (111, 156). Interestingly, brainstem LBD was found in a British patient carrying the same Y1699C mutation (161).

It is currently unknown why the various mutations in the *LRRK2* gene are associated with so different pathologies. One explanation would be that the Lrrk2 protein functions in a pathway upstream of other proteins implicated in the pathogenesis of neurodegeneration. Substrates of Lrrk2 GTPase and kinase activities have to be identified to further understand the molecular mechanisms and how the different pathologies develop.

6.6 LRRK2-mutations in Alzheimer's disease and other neurodegenerative disorders

As discussed above, patients affected by parkinsonism carrying a *LRRK2* mutation generally present clinically with symptoms reminiscent of idiopathic PD. However, some individuals carrying *LRRK2* mutations have exhibited cognitive dysfunction, and co-existing Alzheimer-type pathology has been identified in several *LRRK2* mutation carriers. In addition, tau pathology similar to that found in PSP, has been demonstrated in a mutation carrier (155). This indicated that the Lrrk2 protein could be implicated in the aggregation of misfolded proteins in several neurodegenerative disorders, including AD and other tauopathies, as studied in Paper III and IV.

In Paper III we studied the presence of seven mutations in the *LRRK2* gene in a series of Norwegian patients with dementia. Of the 242 patients, 161 had clinical diagnosis of probable or possible AD, and no mutation carriers were identified. From these results we concluded that *LRRK2* mutations are not a common cause of AD. The study population was limited and only seven mutations were examined. The possibility of other *LRRK2* mutations being present in our series can therefore not be excluded. Extensive sequencing of

the large *LRRK2* gene would have been required to address this question, but this was not possible to perform within the available financial resources. The number of patients with diagnoses other than AD in the study was small. We can therefore not make definitive statements about a possible role of *LRRK2* in these disorders, and we can only conclude that we did not find any mutations in our material.

Another limitation of the study was that all diagnoses were clinical, no neuropathological data were available. Clinical diagnoses of the various dementias are difficult, but have improved by the use of validated diagnostic criteria. The NINCDS-ADRDA criteria used for AD in Paper III have been associated with diagnostic accuracy rates of over 80% (162).

Paper III does not exclude the possibility of other genetic variants within the *LRRK2* gene conferring susceptibility to AD. A locus on chromosome 12 conferring susceptibility to late-onset AD was reported by Pericak-Vance and colleagues (163). Additional evidence for a locus on this chromosome has been reported in subsequent linkage studies (16). In a fine-mapping study of this locus, evidence for linkage was greatest in families where at least one affected individual had a neuropathologic diagnosis of a different form of dementia, dementia with Lewy bodies (DLB) (164). One could therefore speculate that the linked region is a locus for LBD and not AD.

The chromosome 12 locus is large and contains a considerable number of genes, including *LRRK2*, which is located underneath the linkage peak in the study. Studies of the possible association between common genetic variants within *LRRK2* and AD or DLB have so far not been published. However, no association between common genetic variability within the LRRK2 gene has been found for PD in most studies, making it less likely that such an association will exist for AD (165, 166).

In Paper IV, 654 cases with pathologically confirmed AD were screened for the G2019S-mutation, and one mutation carrier was identified. In addition to neurofibrillary pathology, this individual showed Lewy bodies in the amygdala,

but not in brainstem nuclei or cortex. Such amygdala-only Lewy bodies are a relatively common finding in advanced AD (167). One of 156 clinically normal, aged individuals screened for the mutations also carried the G2019S variant. This individual died at the age of 68 and did not have pathology on brain examination. Our interpretation of the finding of a *LRRK2* mutation in a case of AD and a healthy control was that it might have been a coincidental finding and a result of the reduced penetrance of G2019S-associated parkinsonism.

Papers III and IV suggest that *LRRK2* mutations are not frequent in neurodegenerative disorders other than familial and apparently sporadic PD. This finding is now supported by several other studies. Zabetian and colleagues did not find the G2019S mutation in a series of 754 patients with AD, of which histopathological data was available for 47% of the subjects (168). In a screening for the G2019S mutation in patients with PD, AD (n=1444), PSP (n=186) and other neurodegenerative disorders, the mutation was only found in patients with PD (169). Studies of patients with dementia and Parkinson plus syndromes from Asian populations have also failed to identify any mutations (170, 171). Finally, in a paper not included in this thesis we examined a series of 244 cases diagnosed as having PSP on the basis of pathologic findings. None of the individuals carried the studied amino acid substitutions, which included G2019S, and the pleomorphic pathology–associated R1441C/G/H (172).

6.7 PINK1 mutation frequencies and clinical findings

In Paper V we performed an extensive mutational analysis of the *PINK1* gene in a total of 131 Norwegian patients with early-onset or familial late-onset PD. We did not find any homozygous or compound heterozygous mutation carriers. Three patients (2%) carried heterozygous mutations that were not found in any of 350 Norwegian control individuals. The main conclusion of this study is therefore that pathogenic *PINK1* mutations are rare our population.

We cannot completely exclude the presence of unidentified mutations. For example, mutations affecting gene expression localized in the promoter or the introns, would not have been detected in this study. Deletions and

multiplications of the *PINK1* gene seem to be rare, and only one exonic deletion has been reported (94). Our quantitative analysis did not detect any mutations, but we only examined two exons and some mutations might have been missed. However, the *PINK1* gene spans only 1.8 kb of genomic sequence and therefore most deletion and duplication mutations are likely to encompass the entire gene, or are likely to be sufficiently small to be identified through sequence analysis.

In addition, we identified three novel genetic variants that are probably benign polymorphisms. Two of them were silent mutations (Arg279Arg and Asn410Asn). The third variant was a novel exonic c.1745G>T mutation removing the stop codon (Stop582Leu), leading to the translation of nine additional amino acids until the next stop codon occurs. This variant was found in one patient and two controls and has unknown pathogenic significance. We found no association between common genetic variation in the *PINK1* gene and PD in Paper V. This result is in accordance with other studies of Finnish and British populations (173, 174).

The frequency of *PINK1* mutations was lower in our study than in some of the previously published reports, as no patient had disease definitely caused by this gene. There are at least two possible explanations for this finding. First, the frequency of *PINK1*-associated disease varies between populations. Studies of patients with sporadic EOP from Italy have found homozygous mutations in 2-4% of patients (97, 99). A study of Asian patients also found homozygous mutations in 2% of EOP patients (98). On the other hand, only one heterozygous carrier was found in a total of 290 PD patients from Ireland (175).

Second, the mutation frequency in a study depends on the criteria used for inclusion of patients. Patients with *PINK1* mutations have a mean age of disease onset of around 31 years (99). The mean age at onset in our study was considerably higher: 44 years in the EOP group and 64 group in the familial PD group. Also, the highest number of mutation carriers is found in studies of autosomal recessive disease. Only a minority of patients included in Paper V had evidence of autosomal recessive disease transmission.

The clinical presentation of the three patients with heterozygous mutations was very similar to that found in most other families and sporadic cases with *PINK1* mutations (97, 100). All patients have a slowly progressive parkinsonian syndrome; none of them showed any sign of early dementia, psychiatric symptoms or had dystonia at disease onset. Only one of our patients has so far received dopaminergic treatment and this patient showed an excellent and sustained effect of levodopa-treatment. She developed severe dyskinesias, which have been successfully treated with the implantation of an STN-stimulator. Overall the clinical features were relatively benign and indistinguishable from idiopathic PD.

6.8 PINK1 heterozygosity and parkinsonism

The role of single heterozygous *PINK1* mutations in PD is difficult to interpret, but there is growing evidence suggesting that heterozygous mutation carriers might be at increased risk to develop disease. The two novel mutations found in Paper V were absent in a large number of Norwegian control chromosomes, making it unlikely that they are rare polymorphisms. The *PINK1* transcript encodes a protein kinase and localizes to the mitochondria (91). Both mutations are located in the kinase domain and the affected amino acids are highly conserved. Thus, these protein alterations may affect the kinase activity of the protein.

Most other investigators have also found a higher frequency of heterozygous mutations in patients compared to controls. If analyzed combined, two studies from Italy found a significant association between *PINK1* heterozygosity and disease (5% in patients and 1% in controls, $P<0.01$) (99). A recent study of PINK1 mutations in 768 patients with PD and matched controls also found a higher frequency of heterozygous mutations in patients (1.2% vs. 0.4%) (176).

Single PINK1 variants could affect the product of the other allele (dominant negative), act as gain-of-function mutations (dominant) or decrease protein activity to around 50% (haploinsufficiency). In all three models, one should observe a dominant pattern of disease transmission. It is therefore of interest

that in one of the patients identified in Paper V, the Gly411Ser mutation seemed to be transmitted in a pattern compatible with autosomal dominant inheritance.

A previous PET study of unaffected heterozygous *PINK1* mutation carriers showed a significant reduction in ^{18}F-dopa uptake in comparison to controls (101). This suggests that some mutations in heterozygous state cause a decreased striatal dopamine storage capacity, predisposing to late-onset PD. Interestingly, mean age at disease onset of heterozygous carriers has been more than 10 years later than in patients with two mutations in the gene (99). In addition, in a recent study of a large German pedigree six members with heterozygous mutations showed mild signs of PD, although they were all unaware of their signs (177).

An alternative explanation is that other factors act in combination with *PINK1* mutations to cause disease. Digenic inheritance of EOP with mutations in both *DJ-1* and *PINK1* has been reported in a Chinese family, and functional studies in a cell model indicated that the two proteins interact (178). Genetic interactions between *PINK1* and *parkin* have also been reported. Loss of *PINK1* in *Drosophila melanogaster* models lead to defects in mitochondrial function with muscle and dopaminergic neuron degeneration that can be rescued by *parkin* (103-105).

In conclusion, there is several lines of evidence indicating a role of heterozygous PINK1 mutations in PD. However, further sequencing of large series of cases and controls and functional studies are warranted before any definite conclusions can be made.

Conclusions

From the studies presented in this thesis we have gained new and important insights into the genetics of PD:

- First, a single Lrrk2 G2019S mutation is established as the most common known genetic cause of PD. The mutation is present in a range of populations and inherited from a common ancestor. Fascinatingly, mutations in the *LRRK2* gene are inherited in an autosomal dominant fashion with reduced penetrance, as proposed by Henry Mjönes in his study from the 1940's.

- Second, the majority of *LRRK2*-associated patients are clinically indistinguishable from typical sporadic PD. Age of disease onset is variable, but mainly late-onset. This demonstrates that a proportion of late-onset PD has a genetic basis.

- Third, the majority of postmortem cases with *LRRK2* mutations have LBD, as found in sporadic PD.

- Fourth, *LRRK2* mutations are uncommon in other neurodegenerative disorders than PD.

- Finally, *PINK1*-associated PD is rare in Norway, but heterozygous mutations might increase the risk of developing disease. This further highlights the role of genetics in PD.

Following the identification of *LRRK2* mutations, a remarkable paradigm shift has occurred in the field of neurodegeneration regarding the influence of genetics on disease. It is now clear that genetics plays a much more important role in the pathogenesis of PD than previously thought.

The remarkable advances in the genetics of parkinsonism over the last ten years have provided substantial insights into the molecular pathways and

disease mechanisms involved in the development and pathogenesis of the syndrome. Mutations in the *LRRK2* and *PINK1* genes only explain the cause of PD in a minority of patients. However, these findings have identified key disease mechanisms of neurodegeneration and generated hypotheses for studies in cell systems and animal models. It appears that sporadic PD is heterogenous in nature, with multiple and varied causal factors acting upon a number of biological pathways.

With improvements in DNA technology, genetic studies in large groups of families and sporadic PD patients are possible. These ongoing studies, and the identification of novel genes within the other *PARK* loci, will further enhance our knowledge of the cause of PD and direct future research.

Hopefully, genetic research and identification of the pathways involved in the development of PD, can pave the way for new therapeutics. Only when disease progression can be stopped, we will have a real treatment for the disorder James Parkinson described almost two hundred years ago.

8. References

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