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Genetics of Parkinson's Disease in the Finnish Population

Doctoral dissertation

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ABSTRACT

There is much controversy regarding the role of genetics in the etiology of idiopathic PD. The causative genes for familial PD in the Finnish population are unknown. The involvement of complex I, one of the mitochondrial energy generating enzymes of oxidative phosphorylation, in the pathogenesis of PD is well established. However, the role of single mutations in the mitochondrial DNA-encoded complex I (MTND) genes is unclear. We performed familial aggregation and complex segregation analysis (CSA) to analyze the occurrence of PD in families and the mode of inheritance in the Finnish population. The molecular genetic analysis of the α-synuclein and the parkin gene was performed to determine whether mutations in these genes could be detected in Finnish patients with familial PD. The role of mtDNA in PD was investigated by analysis of sequence variation in MTND genes.

A family history was obtained for 268 patients with PD and 210 controls selected from the population of the province of northern Ostrobothnia, Finland. A total of 265 nuclear families were included in a CSA with POINTER. The analysis was first carried out for the total data set, and then the heterogeneity between families with early-onset PD (EOPD, proband under 55 years at onset) and families with late-onset PD (LOPD) was examined. Finally, families with more than one affected individual were analyzed separately. The α-synuclein gene was sequenced and 8 previously described mutations in the parkin gene were screened in 22 unrelated patients with familial PD. In addition, 67 controls and 45 patients with sporadic PD were included in the association analysis of polymorphism of the α-synuclein gene. The nonsynonymous to synonymous substitution ratio (K_a/K_s) of the seven MTND genes was determined from 183 Finns. The differences in K_a/K_s of MTND genes between the European mtDNA haplogroup clusters (HV, JT, KU, IWX) were analyzed using a statistical approach. Altogether 238 patients underwent clinical examination together with haplogroup analysis and the clinical features between patient groups defined by the K_a/K_s ratio were compared.

Ten per cent of the probands reported an affected first degree relative, whereas the corresponding frequency was 3.8 per cent in the controls (p=0.01). The relative risk of PD among the first degree relatives of the patients with PD was 2.9 fold (95 % CI 1.3-6.4) and the cumulative incidence of PD by the age of 90 years was 3.3-fold higher among the first degree relatives of the patients than those of the controls. The results of the CSA strongly rejected the sporadic model. Significant heterogeneity was found between the families with EOPD and LOPD, suggesting that major genes have a greater role in EOPD than in LOPD. The CSA of familial PD supported the hypothesis that a major locus was present in this subset, but it was not possible to distinguish between a recessive model with a high penetrance and a dominant model with lower penetrance. The sequence analysis found no mutations or polymorphism in exons 3-7 of the α-synuclein gene. Sequencing of the non-coding exons 1 and 1', however, revealed three novel alterations in the T10A7 sequence at the 5' end of exon 1'. The frequencies of the exon 1' polymorphic genotypes or alleles between patients with familial PD and control subjects revealed no statistically significant differences. No association for sporadic PD was observed. Screening for the parkin gene revealed no mutations in our patients. The haplogroup clusters differed in the K_a/K_s ratio of the MTND genes, the clusters HV and KU having a lower ratio than clusters JT and IWX. Supercluster JTIWX with a high K_a/K_s ratio was more frequent among PD patients and even more frequent among patients with PD who developed dementia.

The present study provides evidence that familial aggregation of PD exists in the Finnish population and that it is most likely caused by inherited susceptibility. Genetic heterogeneity exists between families with EOPD and LOPD. The results do not support a role of the α-synuclein gene or point mutations in the parkin gene in familial PD in our sample. Our results indicate that a relative excess of nonsynonymous mutations in MTND genes in supercluster JTWIX is associated with increased risk of sporadic PD and progression of the disease to dementia.

National Library of Medicine Classification: WL 359

Medical Subject Headings: Parkinson disease/genetics; mutation; point mutation/genetics; polymorphism (genetics); family; family health; genetic heterogeneity; genetics; population; sequence analysis; DNA, mitochondrial

This book is dedicated to Paavo, Krista and Akseli

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Jaana Autere

ABBREVIATIONS

AAO	Age at onset
AD	Alzheimer's disease
ADPD	Autosomal dominant Parkinson's disease
AR-JP	Autosomal recessive juvenile parkinsonism
ARPD	Autosomal recessive Parkinson's disease
α-synPD	α-synuclein Parkinson's disease
CDR	Clinical dementia rating
CSA	Complex segregation analysis
DZ	Dizygotic twins
DLB	Dementia with Lewy bodies
EOPD	Early onset Parkinson's disease
FPD	Familial Parkinson's disease
K_a/K_s ratio	Nonsynonymous to synonymous substitution ratio
LOPD	Late onset Parkinson's disease
MMSE	Mini Mental State Examination
MZ	Monozygotic twins
MPP+	1-methyl-4-pyridinium
MPTP	N-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MtDNA	Mitochondrial DNA
MTND	Genes in mtDNA encoding subunits of complex I
OXPHOS	Oxidative phosphorylation
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDD	Parkinson's disease with dementia
PET	Positron emission tomography
SN	Substantia nigra
UCH-L1	Ubiquitin C-terminal hydrolase L1
UPDRS	Unified Parkinson's Disease Rating Scale
UPP	Ubiquitin-proteosomal pathway

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by the Roman numerals **I-IV**

I **Jaana Autere,** Jukka Moilanen, Vilho Myllylä, Kari Majamaa. Familial aggregation of Parkinson's disease in a Finnish population. **Journal of Neurology Neurosurgery and Psychiatry** 69;107-109 2000.

II Jukka Moilanen, **Jaana Autere,** Vilho Myllylä, Kari Majamaa. Complex segregation analysis of Parkinson's disease in the Finnish population. **Human Genetics** 108:184-189 2001.

III **Jaana Autere,** Mikko Hiltunen, Arto Mannermaa, Pekka Jäkälä, Päivi Hartikainen, Kari Majamaa, Irina Alafuzoff, Hilkka Soininen. Molecular genetic analysis of the α-synuclein and the parkin gene in Parkinson's disease in Finland. **European Journal of Neurology** 9:479-483 2002.

IV **Jaana Autere,** Jukka Moilanen, Saara Finnilä, Hilkka Soininen, Arto Mannermaa, Päivi Hartikainen, Merja Hallikainen, Kari Majamaa. Mitochondrial DNA polymorphisms in Parkinson's disease. Submitted for publication.

CONTENTS

1. INTRODUCTION

Idiopathic Parkinson's disease (PD) is the second most common neurodegenerative disease. Degeneration of dopaminergic neurons in the substantia nigra (SN) contribute to the typical clinical features of PD: tremor at rest, rigidity, bradykinesia and postural instability. The identification os several genes and loci in familial PD along with experimental studies have afforded substantial new information of the pathogenesis of PD. Although major scientific efforts have been made recently, the causes of idiopathic PD are still largely unknown. There is still much controversy regarding the genetic components of PD, as known genes account for only a minority of PD cases, and the familial distribution of PD in most cases is inconsistent with straightforward Mendelian modes of inheritance. Furthermore, a recent twin study indicated that genetic factors are involved in early onset PD (EOPD), but not in late onset PD (LOPD) (Tanner et al. 1999). One widely accepted theory is that PD in most cases is a complex disease both genetic and environmental factors contributing to susceptibility. However, few specific susceptibility genes or environmental agents have been identified and the relative contribution of genetic versus environmental factors is poorly understood. Although dopaminergic drugs have changed the natural course of PD dramatically, major problems still exist in the long-term treatment of this disabling progressive disease. As therapies that slow down the progression of PD are needed, better understanding of the etiology and pathogenesis of PD is warranted.

The research tools of genetic epidemiology are uniquely suited to deal with the genetic contributions to complex diseases (Morton 1997). When the current study was started, most previous studies on familial aggregation of PD were uncontrolled, or if the were case-control studies they were based on hospital-based samples from specialized movement disorder centers. Only two population-based case-control studies had been conducted before ours (Semchuk et al. 1993, Marder et al. 1996). The need for a segregation analysis of PD was also obvious: none was available at the time when the present study was initiated and one was published when the present study was being conducted (Zareparsi et al. 1998).

The identification of three PD genes, α-synuclein, parkin and ubiquitin C-terminal hydrolase L1 (UCH-L1), supports the role of genetic factors in PD. No molecular

genetic analyses have been conducted in familial PD in Finland before this study. Furthermore, the results of our segregation analysis suggested the possibility of identifying a major gene responsible for PD in Finnish patients with familial PD and encouraged us to start searching of mutations in the α-synuclein gene and parkin gene.

There is also growing evidence that mitochondrial respiratory chain dysfunction may play a role in the degenerative process in PD. Complex I defect in PD is well established, but the mechanisms leading to it remain to be determined. It has been suggested that mitochondrial DNA (mtDNA) mutations are responsible for many sporadic cases of PD (Parker and Swerdlow, 1998). There is some experimental evidence that a mtDNA abnormality contributes to complex I defect in PD (Swerdlow et al. 1996, Swerdlow et al. 1998, Gu et al. 1998). Attempts to examine mtDNA -encoded complex-I genes (MTND genes) for the presence of specific disease-related sequence changes have been inconclusive, and samples used have been small (Ibeke et al. 1995, Bandmann et al. 1997, Kösel et al. 1998, Kösel et al. 2000, Simon et al. 2000, Richter et al. 2002, Vives-Bauza et al. 2002). Recently, two studies have suggested that mtDNA polymorphism may modify the risk of PD (Ross et al. 2003, van der Walt et al. 2003).

The Finnish population is ideal for the study of the genetic component of diseases. The population has evolved from a small number of ancestors who brought a random assortment of disease genes to Finland. As a result of national and regional isolation little further mixing of genes has happened. Therefore, the population has remained genetically homogenous to the present day, but also enriched with rare genetic mutations (Norio et al. 1973, Norio 2003). In addition, the well-documented mtDNA sequence variation in Finns provides a valuable source for analysing mtDNA variation as a risk factor for disease (Finnilä et al. 2001a).

The present study was designed to investigate the genetics of PD in the Finnish population using methods of genetic epidemiology and molecular genetics. Familial aggregation and the role of polygenic or major locus inheritance in PD were examined. Direct sequencing of the α-synuclein and screening for eight point mutations in the parkin gene was performed from patients with familial PD. Furthermore, the contribution of mtDNA sequence variation in MTND genes as a risk factor for sporadic PD was analyzed.

2. REVIEW OF LITERATURE

2.1. Parkinson's disease

2.1.1. Epidemiology

Parkinson's disease (PD) was first described by James Parkinson in his essay on the shaking palsy published in 1817 (Parkinson 1817). Nowadays it is the second most common neurodegenerative disease after Alzheimer's disease (AD). The incidence of PD is 16-19/100 000/year, according to the data from a recent review including best incidence studies with similar methodologies and comparing the results on standardised populations (Twelves et al. 2003). This comparison included two studies from south-western Finland which also showed that the age-adjusted prevalence of PD had increased from 139 per 100 000 population in 1971 to 166 per 100 000 population in 1992 (Marttila and Rinne 1976, Kuopio et al. 1999). In most studies, the peak incidence of PD is between 70 and 79 years of age and the mean symptom onset is 60-65 years of age (Twelves et al. 2003). The age-adjusted prevalence of PD has been found to increase with age from 60 per 100 000 population for those aged 65 to 69 years to 350 per 100 000 population for those aged 85 to 89 years (de Rijk et al. 1997).

Recently, Elbaz et al. (2002) estimated that the lifetime risk of developing PD is 2 percent for men and 1.3 percent for women. Male preponderance is also observed in other studies with respect to the prevalence and incidence rates (Mayex et al. 1995, Fall et al. 1996, Kuopio et al. 1999, Baldereschi et al. 2000). While the etiology of PD is poorly understood the explanation for the increased risk of PD in male subjects is also unknown.

Although knowledge about ethnic and geographical differences in the prevalence of PD is scanty due to the lack of comparable epidemiological studies, it is known that PD occurs in all ethnic groups around the world and shows geographical variation the prevalence being lowest in China, Japan and Africa (Zhang and Roman 1993). The prevalence of PD seems to be similar across European countries (de Rijk et al. 1997). The factors responsible for geographic variation in the occurrence of PD are unknown, but mainly environmental factors have been suggested (Zhang and Roman 1993). The genetic component in PD is the alternative explanation which is supported by the

reported higher age-adjusted prevalence of PD among Caucasians and Hispanics than among African Americans in the US population (Mayeux et al. 1995).

2.1.2. Pathological and clinical features

The pathological markers of PD are selective degeneration of dopaminergic neurones in the SN pars compacta and the formation of fibrillar cytoplasmic inclusions, Lewy bodies, in the remaining neurones. Lewy bodies are also seen in other prediction sites than the SN, especially the nucleus basalis of Meynert, dorsal motor nucleus of the vagus, hypothalamus, and the locus coeruleus. For pathological diagnosis, the SN degeneration and the Lewy bodies are required in the setting of typical clinical signs (Forno 1996). The neuronal cell loss is already well advanced when diagnosis is made, since it is estimated that by the time of symptoms appear striatal dopamine levels have decreased by 80 percent. Immunocytochemical studies performed with monoclonal antibodies to α-synuclein have shown that the α-synuclein is a component of Lewy bodies (Spillantini et al. 1997, Wakabayashi et al. 1997, Baba et al. 1998, Spillantini et al. 1998). Parkinsonism or Parkinsonian syndrome are the terms used for a clinical disorder characterized by at least two of the following motor manifestations: tremor, muscular rigidity and bradykinesia. Idiopathic PD accounts for approximately 85 percent of all cases with parkinsonism. PD is progressive and usually has an insidious onset in mid to late adulthood. The diagnosis of PD is primarily clinical based on the presence of typical motor manifestations and exclusion of other conditions causing parkinsonism. The most widely used criteria for PD are those of the United Kingdom Parkinson's Disease Society Brain Bank criteria (Daniel and Lees 1993). It has been shown that only about 75 percent of clinical diagnoses of PD are confirmed at autopsy (Rajput et al. 1991, Hughes et al. 1992). This is largely explained by the fact that cardinal signs of parkinsonism also occurs in other disorders such as dementia with Lewy bodies (DLB), parkinsonism caused by neuroleptic drugs, vascular degeneration with lacunar infarcts in basal brain and atypical parkinsonism in progressive supranuclear palsy (PSP), multiple system atrophy (MSA), and corticobasal degeneration (CBD). Features suggesting atypical parkinsonism are rapid progression of parkinsonism, and poor or transient response to levodopa therapy, or atypical signs such as supranuclear gaze palsy, early postural instability, early autonomic failure, or

pyramidal or cerebellar signs. These disorders most likely result from loss or dysfunction of the dopaminergic neurons in the SN, but may or may not have Lewy bodies on pathology. To improve diagnostic accuracy, new diagnostic criteria for PD have been proposed (Gelb et al. 1999). These criteria emphasize the asymmetric onset of symptoms as a supportive feature for PD, and differentiate three levels of diagnostic confidence: definite, probable and possible.

2.1.3. Environmental risk factors

The observation that the toxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) can cause nigrostriatal dopaminergic degeneration and parkinsonism has called attention to environmental agents as a risk factors for PD (Nicklas and Heikkilä 1985). A number of agricultural chemicals are similar in structure to MPTP, and epidemiological studies have provided evidence that chronic pesticide exposure increases the risk of PD (Tanner et al. 1989, Hubble et al. 1993, Semchuk et al. 1993, Betarbet et al. 2000). Pesticide exposure is probably responsible for an increase in the age-adjusted incidence of PD among subjects who have worked for more than a decade on a plantation (Petrovitch et al. 2002). Living in rural areas and drinking well water have also been suggested to increase the risk of PD (Koller et al. 1990). In accordance with a possible environmental causative factor, a very significant male and a significant rural predominance was observed in a Finnish epidemiological study (Kuopio et al. 1999). Head trauma is a risk factor for PD (Semchuk et al. 1993, Taylor et al. 1999) and associated with a 3-year younger age at onset (Maher et al. 2002). Cigarette smoking and intake of coffee or caffeine have been shown to be associated with decreased risk of PD (Tzourio et al. 1997, Gorell et al. 1999, Preux et al. 2000, Tanner et al. 2002). The environmental factors in early life in the etiology of PD have also been proposed, but no clear risk factors for PD have been found (Martyn et al. 1995).

2.2. Genetics of Parkinson's disease

2.2.1. Genetic epidemiology

Familial aggregation Using multivariate statistical methods, a positive family history of PD has been shown to be the strongest risk factor for the disease (Semchuk et al. 1993, Preux et al. 2000). In another study using the same methods, the family history

of a neurological disease (PD, AD, dementia and tremor) was the second most powerful predictor for PD after pesticide use (Hubble et al. 1993). Comparison of the results of these studies is impossible, since the latter study did not differentiate the family history of PD from other neurological diseases. Uncontrolled studies have demonstrated that 13 to 27.5 percent of patients with PD report having an affected first- or second-degree relative and 10 to 16 percent of patients with PD report having an affected first-degree relative (Lazzarini et al. 1994, Uitti et al. 1997). Lazzarini et al. (1994) estimated that the cumulative risk of PD by the age 90 may exceed 50 percent in probands' siblings when a parent is also affected. As shown in Table 1, several case-control studies regarding familial aggregation of PD have been conducted during recent years. Four of these studies represent the best attempt at investigating familial aggregation of PD, since they are based on population-based materials and obtain valid estimates of the risk ratios with confidence intervals (Semchuk et al. 1993, Marder et al. 1996, Elbaz et al. 1999, Kuopio et al. 2001). A two- to threefold increased risk of PD in first-degree relatives of affected individuals compared with first-degree relatives of unaffected individuals has been found in all these studies. Higher risks for familial PD (OR 7.1-14.6) were found in case-control studies in Italy by DeMichele et al. (1995) and in Germany by Vieregge et al. (1994) but the inclusion of first- and second-degree relatives and patients of movement disorders clinics may account for these results.

One case-control study also indicates that among first-degree relatives of PD patients, men are at 1.9 times higher risk than women of developing PD (Marder et al. 1996). When considering different ethnic backgrounds, Caucasians appear to be at increased risk compared with African Americans and Hispanics (Marder et al. 1996). The familial clustering of PD seems to be stronger for early-onset PD compared with late-onset disease. An interesting study by Payami et al. (2002) established that the age-specific risk of PD is increased eightfold among relatives of patients with early-onset disease and threefold among relatives of late-onset disease.

An epidemiologic study using an Icelandic genealogical database in relation to encrypted medical information about a population-based group of patients examined the issue of genetic and environmental contributions to PD (Sveinbjornsdottir et al. 2000). The study led to the identification of many pedigrees containing two or more related patients with PD. One large pedigree contained 44 patients with EOPD or LOPD from a

common founder. The demonstration that the familial clustering of PD extends beyond the nuclear family provides evidence that the disease has a genetic component. The importance of the genetic component in PD is also supported by a large study of sibling pairs with, where siblings showed greater similarity in the age at onset than in the year of onset (Maher et al. 2002).

Table 1. Case-control studies of familial aggregation of PD.

Study	*N (PD patients/ controls)*	*% affected first-degree relatives (PD patients/ controls)*	*Odds ratio (95% CI)*	*Study population*
Semchuk et al 1993	130/260	10.9/5.1	2.2 (0.9-5.3)	Population-based, Canada
Payami et al. 1994	114/114	16/4	3.5 (1.3-9.4)	Clinic-based, USA
Morano et al. 1994	74/148	16.2/4.7	NA	Clinic-based, Spain
Bonifati et al. 1995	100/100	24/6	5.0 (2.1-11.9)	Clinic-based, Italy
Marder et al. 1996	233/1172	NA	2.1 (1.2-3.9)	Population-based, USA
Elbaz et al. 1999	175/481	10.3/3.5	3.2 (1.6–6.6)	Population-based, Europe[a]
Taylor et al. 1999	140/151	18.6/6.8	6.1 (2.4-15.6)	Clinic-based, USA
Preux et al. 2000	140/280	10.7/2.5	9.3 (2.6-32.6)	Clinic-based, France
Kuopio et al. 2001	119/238	12.6/4.6	2.7 (1.3-5.9)	Population-based, Finland
Kurz et al. 2003	245/100[b]/100[c]	12.2/5/3	NA	Population-based, Norway

a includes five population-based studies from France, Italy, the Netherlands and Spain,

b control group includes patients with diabetes mellitus

c control group include healthy individuals

NA not available

The mode of inheritance The mode of inheritance in most PD families is unknown. Segregation ratios on families with multiple affected members are compatible with an autosomal dominant rather than a recessive mode of inheritance among PD

families (Maraganore et al. 1991, Lazzarini et al. 1994, Bonifati et al. 1995, DeMichele et al. 1995, Plante-Bordeneuve et al. 1995). Age at onset data in several studies have been consistent with genetic anticipation and suggest the possible involvement of an unstable trinucleotide repeat (Payami et al. 1995, Plante-Bordeneuve et al. 1995, Maraganore et al. 1996). The age at onset of affected family members differed significantly between generations and was earlier in the proband generation than in the parental generation. Maternal inheritance of PD in some cases has been observed indicating a mtDNA contribution in PD (Swerdlow et al. 2001, Wooten et al. 1997). Contradictory results have been found in another study where secondary cases were observed significantly more frequent in the paternal than in the maternal line in the study of (DeMichele et al. 1995).

To date, apart from our study only two segregation analyses have been conducted. Both these studies were conducted with a clinic population. Zareparsi et al. (1998) performed complex segregation analyses using kindreds of 136 Parkinson's disease patients randomly selected from a clinic population. The hypotheses of a nontransmissible environmental factor, a major gene or type (sporadic), and all Mendelian inheritance (dominant, recessive, additive, decreasing) were rejected in the analysis. The investigators concluded that familial clustering of PD in this data set was best explained by a rare familial factor which is transmitted in a nonmendelian fashion and influences the age at onset of PD. More recently, another segregation analysis by Maher et al. (2002) analyzed 948 nuclear families in an age-at-onset model and a susceptibility model. The first model found evidence for a major gene influencing age at onset rather than susceptibility. The second model found evidence for a Mendelian gene influencing susceptibility. In both models, the 'no major gene' and environmental models were rejected.

Twin studies Twin studies do not support the existence of a genetic factor in the etiology of PD, since the concordance rates for monozygotic twins (MZ) are not higher than the concordance rates for dizygotic pairs (DZ) (Duvoisin et al. 1981, Ward et al. 1983, Marttila et al. 1988, Vieregge et al. 1992, Tanner et al. 1999, Vieregge et al. 1999). However, in a subset of twins with PD at or before age 50 years in at least 1 twin, MZ concordance is 1.0 and DZ concordance is 0.167 (Tanner et al. 1999). The investigators suggest that genetic factors are important in EOPD, but not in LOPD.

However, it is important to draw attention to the limitations of twin studies on PD. These studies are cross-sectional, with the exception of the study of Vieregge et al. (1999), which has 8 years follow-up. The major problem in twin studies of PD is that they are unable to detect subclinical PD and therefore may underestimate MZ concordance for the disease. PD may develop later in the second twin or the second twin may die of another cause before the onset of symptoms. The possibility of asymptomatic twin pairs was taken into account in the study by Piccini et al. (1999), establishing that concordance levels for dopaminergic dysfunction identified by [^{18}F] dopa positron emission tomography (PET) were significantly higher in MZ than DZ twin pairs with no apparent history of familial aggregation of PD. Furthermore, the level of concordance in MZ pairs increased with time. In agreement with these results, a clinical test battery examining motor function, olfaction and mood was abnormal in a greater proportion of asymptomatic first-degree relatives of PD patients than in controls (Montgomery et al. 1999).

2.2.2. Molecular genetics of familial PD

Over the last six years, four genes causing familial PD have been discovered: α-synuclein, parkin, UCH-L1 and DJ-1 (Polymeropoulos et al. 1997, Kitada et al. 1998, Leroy et al. 1998, Bonifati et al. 2003). In addition, linkage studies have identified five chromosomal loci with evidence of PD-related genes (Gasser et al. 1998, Farrer et al. 1999, Valente et al. 2001, van Duijn et al. 2001, Funayama et al. 2002, Hicks et al. 2002). These genes and chromosomal loci are summarised in Table 2.

ADPD The Contursi kindred from Italy was the first large family with PD reported with autopsy confirmation (Golbe et al. 1990, Golbe et al. 1996). At the time this kindred was reported, 60 individuals in 5 generations were affected with PD and the inheritance pattern was apparently autosomal dominant. The mean AAO of PD was 45.6 years (SD 13.5, range 20-85). A young AAO and rapid rate of progression distinguish PD in this kindred from that in the community. Otherwise the clinical features were similar to those in typical PD: tremor occurred frequently and the patients responded well to levodopa. The pathological findings were also typical of those in PD including Lewy bodies.

Table 2. Genes and loci associated with inherited PD

Gene	Locus	Clinical phenotype and pathology	Reference
α-synuclein (PARK1)	4q21-q23	ADPD, typical PD, early AAO, rapid progression, typical PD pathology	Polymeropoulos et al. 1997
Parkin (PARK2)	6q25.2-q27	AR-JP and ARPD, early AAO, slow progression, dystonia, L-dopa induced dyskinesias, hyperreflexia, no Lewy bodies	Kitada et al. 1998
UCH-L1(PARK5)	4p14-15.1	ADPD, typical PD, AAO 49-51 y	Leroy et al. 1998
unknown (PARK3)	2p13	ADPD, low penetrance, typical PD, dementia, AAO 36-89 y, typical PD pathology, AD changes in some cases	Gasser et al. 1998
unknown (PARK4)	4p15	ADPD and DLB, early AAO, typical PD pathology, DLB pathology	Farrer et al. 1999a
unknown (PARK6)	1p35-36	ARPD; typical PD, early AAO (mean 42 y), pathology unknown	Valente et al.2001, 2002
DJ1 (PARK7)	1p35-36	ARPD, early AAO (40 y or under), hyperreflexia, pathology unknown	van Duijn et al. 2001, Bonifati et al. 2003
unknown (PARK8)	12p11.2-q13.11	ADPD, low penetrance, early AAO (mean 51 y), typical PD, no Lewy bodies	Funayama et al. 2002
unknown (PARK10)	1p32	Classic LOPD, mean AAO 66 y	Hicks et al. 2002

Abbreviations: AAO, age at onset; ADPD, autosomal dominant Parkinson's disease; ARPD, autosomal recessive Parkinson's disease; DLB, dementia with Lewy bodies.

α-synuclein gene Polymeropoulos et al. (1996) identified genetic linkage at chromosome 4q21-q23 to the early-onset form of PD in the Contursi kindred. Soon after, the Ala53Thr mutation in the α-synuclein gene was reported to segregate with PD in the Contursi kindred and three families of Greek origin (Polymeropoulos et al. 1997). The same mutation was subsequently identified in two Greek families with ADPD (Papadimitriou et al. 1999). In both families, asymptomatic carriers older than the expected AAO were found suggesting the possibility of incomplete penetrance. As in

the Contursi kindred, the clinical phenotype in patients with α-synuclein PD (α-synPD) living in Greece included younger AAO compared with sporadic PD (Papapetropoulos et al. 2001). Typical features of Greek patients with α-synPD were also low frequency of tremor at onset of symptoms and longer duration of disease. Most Greek α-synPD families originate from a small geographic area, and there has been migration between Greece and Southern Italy for hundreds of years. These points together with haplotype analyses suggest that the Ala53Thr mutation might be a founder mutation (Papadimitriou et al. 1999). A second mutation in the α-synuclein gene, Ala30Pro mutation, has been described in a family of German origin (Krüger et al. 1998). Since then, several groups around the world have failed to find mutations in α-synuclein gene in familial or sporadic PD (Munoz et al. 1997, Chan et al. 1998, Farrer et al. 1998, Parsian et al. 1998, Vaughan et al. 1998, Wang et al. 1998, Warner and Schapira 1998). Although the mutations in the α-synuclein gene have now been proved to be rare, the discovery of these mutations provided an important clue for research into the pathogenesis of PD. The importance of the α-synuclein will be discussed below on in the section dealing with the pathogenesis of PD.

Other ADPD genes and loci After the discovery of the α-synuclein mutations one causative gene and three linked loci for ADPD have been identified. A genetic locus on 2p13 (PARK3) was identified in linkage analysis of families with multiple affected individuals with parkinsonism closely resembling sporadic PD, including similar mean AAO (Gasser et al. 1998). However, several members of chromosome 2-linked families have shown signs of dementia, and neuropathology has revealed the presence of neurofibrillary tangles and Alzheimer plaques in addition to neuronal loss in the SN and typical Lewy bodies (Gasser 2001). Further study genotyping the PARK3 locus with microsatellite markers in two families allowed the location to be narrowed down (West et al. 2001). No pathogenic mutations have been detected in sequence analysis of 14 known genes within this region and the responsible gene remains to be identified (West et al. 2001).

Linkage analysis by Farrer et al. (1999a) identified a chromosome 4p haplotype (PARK4) to segregate with the disease in a large family called the Iowa kindred, which was previously described as two separate families (Waters and Miller 1994, Muenter et al. 1998). Before linkage analysis mutations in the α-synuclein gene were excluded in

this pedigree (Farrer et al. 1998). Phenotypic variability is observed in this kindred clinical diagnoses varying from PD to psychosis. The pedigree also included individuals with postural tremor sharing the same haptotype. Clinical and neuropathological findings of one affected family member supported the diagnosis of DLB (Gwinn-Hardy et al. 2000).

A missense mutation in the gene for UCH- L1 (PARK5) has been identified in two patients from a German PD family (Leroy et al. 1998). This mutation, Ile93Met causes impaired proteolytic activity of UCH-L1 leading to abnormal aggregation of proteins in the brain. The phenotype of mutation is typical for PD with AAO 49-51 years of age, presence of tremor and good response to levodopa. The Ile93Met mutation seems to be rare and was not found in another study performed on 96 Caucasian families with PD (Harhangi et al. 1999). However, the importance of UCH-L1 in the etiology of PD is further suggested by the finding that polymorphisms in UCH-L1 gene might protect against PD (Maraganore et al. 1999).

Genome wide linkage analysis on a large Japanese family with ADPD has identified a locus for disease on chromosome 12p11.2-q13.1 (PARK8) (Funayama et al. 2002). The phenotype of disease is comparable with idiopathic PD except for early age at onset (mean 51 years). Neuropathological examination has demonstrated nigral degeneration without Lewy bodies. Low penetrance is observed in the ADPD family linked to this locus, as in all ADPD families linked to other loci (PARK3, PARK4 and PARK5).

AR-JP, ARPD and parkin gene The genetic locus for autosomal recessive juvenile parkinsonism (AR-JP) was mapped to chromosome 6q25.2-q27 in the Japanese population (Matsumine et al. 1997). Different deletions including single (Ex4Del) and multiple exonic deletions (Ex3-7Del) in a novel gene called parkin were described in 16 Japanese families with AR-JP (Hattori et al. 1998a, Kitada et al. 1998). The parkin gene (PARK2) consists of 12 exons with an open reading frame of 1395 bp. Characteristic clinical features for patients with AR-JP were the mean age at onset of 24.6 years (range 8-43 years), levodopa responsive parkinsonism, diurnal fluctuations, early and severe levodopa induced motor fluctuations and dyskinesias, and increased tendon reflexes (Kitada et al. 1998). Some dystonic features were also frequently seen. Pathological

examination show selective degeneration of SN dopaminergic neurons without Lewy bodies. A linkage study carried out in European families and in an Algerian family with AR-JP disclosed linkage to PARK2 locus demonstrate that parkinsonism linked to this locus is not restricted to the Japanese population (Tassin et al. 1998). The age at onset (mean 35±11 years; range 7-58) in European and Algerian patients from the PARK2 locus-linked families was older than that in Japanese patients (Tassin et al. 1998). Mutation analyses detected additional deletions in the parkin gene from two French families, and one Portuguese and one Algerian family (Lücking et al. 1998). Clinically, families with deletions and without deletions showed no significant difference in the mean AAO (29±16 vs. 35±9years) or disease severity.

The first pathogenic point mutations (Thr240Arg, Gln311Stop) in the parkin gene were found from two Turkish families with AR-JP (Hattori et al. 1998b). In an European study by Abbas et al. (1999) exon 4 deletion was identified in one Italian family and novel point mutations were found from Italian, British, French and German sibling pairs with PD. Point mutations observed in eight families included three frameshift mutations (Gln34/Stop37, Asn52/Stop81, Trp74/Stop81) and five single base pair substitutions (Lys161Asn, Thr415Asn, Arg256Cys, Arg275Trp, Trp453Stop). The clinical features of parkinsonism were indistinguishable from those of idiopathic PD except for earlier age at onset. The mean age at onset in these patients was 39±12 years, but onset up to age 58 was observed. Among mainly European families with ARPD mutations in the parkin gene were found to be causative for the disease in almost half the families (Lücking et al. 2000). This study showed that the mean AAO was significantly younger in patients with familial PD- carrying mutations (34±10 years) than in patients with familial PD without mutations (43±12 years). The patients with parkin mutations showed higher frequency of dystonia, symmetric onset of symptoms, hyperreflexia and better response to levodopa, but were also more likely to have dyskinesia during treatment. A notable finding was that there was no phenotypic difference between patients with missense mutations and those with truncating mutations. Subsequently, several groups have examined parkin gene in the samples of EOPD and found different pathogenic mutations including point mutations as well as exon rearrangements, both deletions and duplications (Jeon et al. 2001, Nisipeanu et al. 2001, Hedrich et al. 2002, Hoenicka et al. 2002).

However, only two studies have included patients with LOPD with an age at onset greater than 45 or 50 years (Oliveri et al. 2001, Foroud et al. 2003). The first of the two studies failed to detect pathogenic mutations in the parkin gene in 23 familial and 95 sporadic patients with an AAO of PD after age 45 years (Oliveri et al. 2001). Gene dosage analysis was not performed in this study and this could have limited the ability to detect heterozygous exon rearrangements (Hedrich et al. 2001, Kann et al. 2002). More recently, the second study using both gene sequencing and dosage studies in patients with familial PD identified parkin mutations in 62 percent of patients with AAO less than 50 years and in 11.2 percent of patients with AAO 50 years or later (Foroud et al. 2003).

Haplotype analysis in 48 European families with EOPD showed that the patients carried 14 distinct mutations, and each mutation was detected in more than 1 family. The results support the hypothesis that exon rearrangements occur independently and recurrently, whereas some point mutations found in families from different geographic origins may have been transmitted from a common founder (Periquet et al. 2001).

Other ARPD loci Two additional loci (PARK 6 and PARK7) for early-onset ARPD have been localized to independent regions on chromosome 1p. Linkage to PARK6 locus was revealed in a large Sicilian family with EOPD (Valente et al. 2001) and in eight families with parkin-negative ARPD from four different European countries (Valente et al. 2002). The study of van Duijn et al. (2001) also disclosed linkage to chromosome 1p in a consanguineous family segregating EOPD. On the basis of further analyses the region was clearly separated more centromeric from PARK6 locus. Mutations in the DJ-1gene (PARK7) have been shown to associate with PARK7 and cosegregate with the disease in an Italian and a Dutch family with an early-onset ARPD (Bonifati et al. 2003). Although the function of DJ-1, a ubiquitously expressed and highly conserved protein, is unknown, there is indirect evidence that it is involved in the cell's response to oxidative stress.

Familial PD (FPD) without defined inheritance pattern In addition to linkage analyses of large families with clear Mendelian inheritance, genome scans have been carried out in large samples of siblingpairs with PD (DeStefano et al. 2001, Scott et al. 2001, Pankratz et al. 2002) or multiple extended pedigrees of PD patients (Hicks et al. 2001). The use of extended pedigrees across a whole population has proved the most

successful strategy (Hicks et al. 2002). Icelandic investigators disclosed a susceptibility locus for LOPD in chromosome 1p32 (PARK10) with lod score 4.9, and suggested that the gene at this locus alone can account for a substantial fraction of the familial aggregation of LOPD in this population (Sveinbjornsdottir et al. 2000). The GenePD study was conducted in a sample of 113 PD-affected sibling pairs and revealed a maximum lod score of 1.30 on chromosome 9 and three other areas on chromosomes 1, 10 and 16 with lod scores ranging from 0.93 to 1.20 (DeStefano et al. 2001). A second linkage analysis in a sample of 174 families with several individuals with PD identified three regions suggestive of linkage on chromosomes 5, 8 and 17 with multipoint lod scores from 1.5 to 2.22 (Scott et al. 2001). Analysis of families with at least one EOPD individual with onset younger than 40 years resulted in strong evidence of linkage to a marker located in the parkin gene, in which mutations were found in 11/18 families. In families with LOPD, lod scores 1.5-2.5 for chromosomes 5, 8, 9, 14, 17 and X were found. The strongest evidence for linkage was a marker on chromosome 17 located about 8 cM from the tau gene. Further studies using the genomic convergence approach succeed in identifying 402 genes which are located within these large genomic linkage regions and expressed in normal SN (Hauser et al. 2003). The linkage study reported by Pankratz et al. (2002) attempted to reduce genetic heterogeneity by excluding families with parkin mutations from genome scan analyses. They reported the strongest linkage for chromosome X. In addition to these analyses, linkage analyses to identify genes influencing AAO of PD have also been conducted. The results of genomic screens for age at onset in AD and PD demonstrate strong linkage evidence on chromosome 1p with lod score 3.4. In addition, evidence for AAO linkage on chromosomes 6 and 10 has been identified in both AD and PD samples (Li et al. 2002). Another genome scan identified four loci with suggestive evidence for linkage to AAO (DeStefano et al. 2002). The strongest evidence of linkage with lod score 2.4 was found on chromosome 2 overlapping with the PARK3 location.

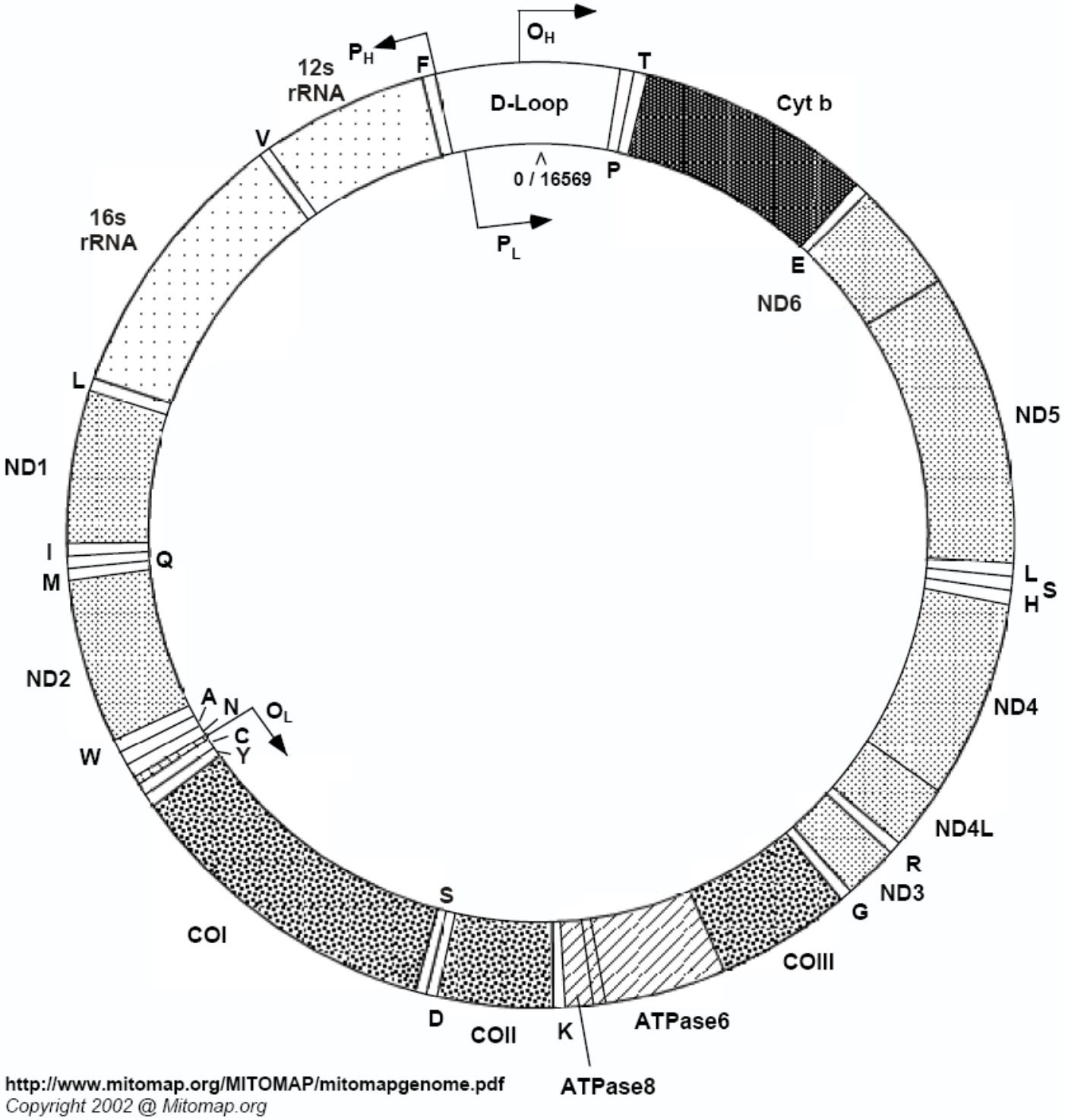
A number of association studies focused on familial PD have been performed. Le et al. (2003) recently found two mutations in the NR4A2 gene affecting 10 of 107 patients with FPD. Mutations were not found from patients with sporadic PD or controls. NR4A2 encodes a member of the receptor superfamily and is essential for the differentiation of nigral dopaminergic neurons. The evidence of linkage in LOPD to a

region on chromosome 17q21 containing the tau gene by Scott et al. (2001) was the reason to study tau as a candidate gene for PD. Martin et al. (2001) carried out family-based tests of association with the tau gene among 426 affected and 579 unaffected subjects from 235 families. They found an association between markers within the tau gene and PD providing further evidence that the tau might be a susceptibility gene for common idiopathic PD.

2.2.3. Mitochondrial genetics in PD

The mitochondrial DNA (mtDNA) is a 16 569 nucleotide pair, closed circular molecule consisting of 13 structural genes, 2 rRNA genes and 22 tRNA genes (Figure 1). Structural genes encode 13 polypeptides, all of which are essential subunits for the mitochondrial energy-generating enzyme complexes of oxidative phosphorylation (OXPHOS). Seven of those genes (MTND1-MTND6, MTND4L) encode subunits of complex I and the remaining subunits are encoded by nuclear DNA (Smeitink and van den Heuvel 1999). Human mtDNA is strictly maternally inherited. However, mitochondrial disease can occur sporadically, which is explained by unique features of mitochondrial genetics such as heteroplasmy, replicative segregation and the threshold effect.

The mtDNA sequence has evolved by the sequential accumulation of base substitutions as people migrated from Africa to various continents. This is seen today as continent-specific mtDNA sequence polymorphisms which are associated with specific mtDNA haplotypes (Wallace et al. 1999). European mtDNAs have been classified into nine mtDNA haplogroups (H, V, U, K, T, J, W, I, and X) (Torroni et al. 1996). Recently Finnilä et al. (2001a) constructed a phylogenetic network for European mtDNA based on the complete nucleotide sequence in the coding region of mtDNA from 192 Finns. As the samples represented all the European haplogroups, the network enables the relationships between the mtDNA haplogroups to be analyzed. The polymorphisms in the coding region yielded a topology that show distinct clusters of haplogroups HV, UK, TJ and WIX (Finnilä et al. 2001a).



http://www.mitomap.org/MITOMAP/mitomapgenome.pdf

Complex I genes (NADH dehydrogenase)

Complex III genes (ubiquinol : cytochrome c oxidoreductase)

Transfer RNA genes

Complex IV genes (cytochrome c oxidase)

Complex V genes (ATP synthase)

Ribosomal RNA genes

Figure 1. Mitochondrial DNA.

The discovery of NADH:ubiquinone oxidoreductase (complex I) dysfunction (Parker et al. 1989, Schapira et al. 1990, Shoffner et al. 1991) led to hypothesis that mtDNA might play a role in the pathogenesis of PD. This hypothesis is supported by genomic transplantation (cybrid) studies where human neuroblastoma cells containing no mtDNA are repopulated with mitochondria from the platelets of patients with PD and controls (Swerdlow et al. 1996, Swerdlow et al. 1998). PD cybrids showed a decrease in complex I activity, an increase in the production of ROS and increased susceptibility to 1-methyl-4-pyridinium (MPP+) -induced apoptosis. These results suggest that the complex I defect in sporadic PD is genetic and most likely arises from mtDNA mutations. Gu et al. (1998) reproduced these findings using the same technique but focused the study on PD patients selected for their low platelet complex I activity. In contrast to these results, Aomi et al. (2001) found the variation of enzyme activities in the cybrid clones to be irrespective of whether their mtDNA was transferred from normal subjects or PD patients. The authors concluded that mtDNA mutations responsible for inducing the complex I reduction should be polymorphic rather than pathogenic.

Several groups have attempted to find mtDNA mutations associated with PD. The presence of the 5kb common deletion in muscle and brain in PD patients have been detected, but the same deletion is found in age-matched controls as well, and this seems to be somatic mutation associated with the aging process (Ibeke et al. 1990, Mann et al. 1992, DiDonato et al. 1993). Shoffner et al. (1993) identified an mtDNA variant 4336A>G at the $tRNA^{Glu}$ gene associated with AD and PD. Since then, the association of 4336>G with PD has been tested in many studies and the meta-analysis of published data suggest that the association is significant (Tan et al. 2000). However, our recent data together with results of Bandmann et al. (1997), Simon et al. (2000) and Richter et al. (2002) do not support the association of 4336A>G with PD (Finnilä et al. 2001b). Our data disclosed that this mutation is uncommon in the Finnish population, as the frequency of this mutation among controls was only 0.63 percent (Finnilä et al. 2001b).

Although a number of single nucleotide polymorphisms in mtDNA-genes have been found in patients with PD, none of these has been shown to account for the complex I deficiency or to be pathogenic (Ibeke et al. 1995, Bandmann et al. 1997, Kösel et al. 1998, Kösel et al. 2000, Simon et al. 2000, Richter et al. 2002, Vives-Bauza

et al. 2002). The total sequence data of mtDNA has revealed distinct clustering of point mutations among two patients with PD (Ozawa et al. 1991). Richter et al. (2002) found novel homoplasmic base changes when they analyzed the mitochondrial MTND1 and MTND2 genes of 10 substantia nigra and 85 platelet samples from PD patients. Grasbon-Frodl et al. (1999) reported two homoplasmic point mutations in the mitochondrial tRNA genes found from 2 patients with PD but not from controls. Recently, association of mitochondrial polymorphisms and PD was investigated in a large case-control study where 10 polymorphisms defining the European mtDNA haplogroups were determined (van der Walt et al. 2003). The risk of PD was significantly reduced in individuals belonging to the J and K haplogroups and carrying the 10398G polymorphism defining these two haplogroups. In contrast, another study suggested an increased risk of PD to be associated with the haplogroup cluster JT (Ross et al. 2003).

2.3. Pathogenesis of Parkinson's disease

2.3.1. α -synuclein and ubiquitin-proteosomal pathway

Understanding of the central role of α-synuclein in PD came from the discovery that filamentous α-synuclein is the major component of Lewy bodies not only in patients carrying mutations in the α-synuclein gene but also in patients with sporadic PD (Spillantini et al. 1997, Baba et al. 1998, Spillantini et al. 1998, Wakabayashi et al. 1997). Since then the mechanisms of accumulation of α-synuclein have been the subject of active research. It is a relatively small protein, expressed in many parts of the brain and localized mostly in presynaptic nerve terminals. The protein was not unknown to investigators of AD at the time when mutations of the α-synuclein gene associated with ADPD were discovered. A fragment of α-synuclein had been shown to be a component of the amyloid plaque in AD, the so-called "non-amyloid component of plaques", NACP (Ueda et al. 1993). Its main function is poorly understood, but it is known to have a role in synaptic plasticity (George et al. 1995) and in the regulation of dopamine vesicle release (Abeliovich et al. 2000). Expression of the α-synuclein gene is observed in brain regions where Lewy bodies have been found in PD and DLB (Solano et al. 2000). In vitro-studies have shown that α-synuclein can polymerize into filaments resembling Lewy bodies, fibrillization is accelerated by pathogenic mutations (Conway

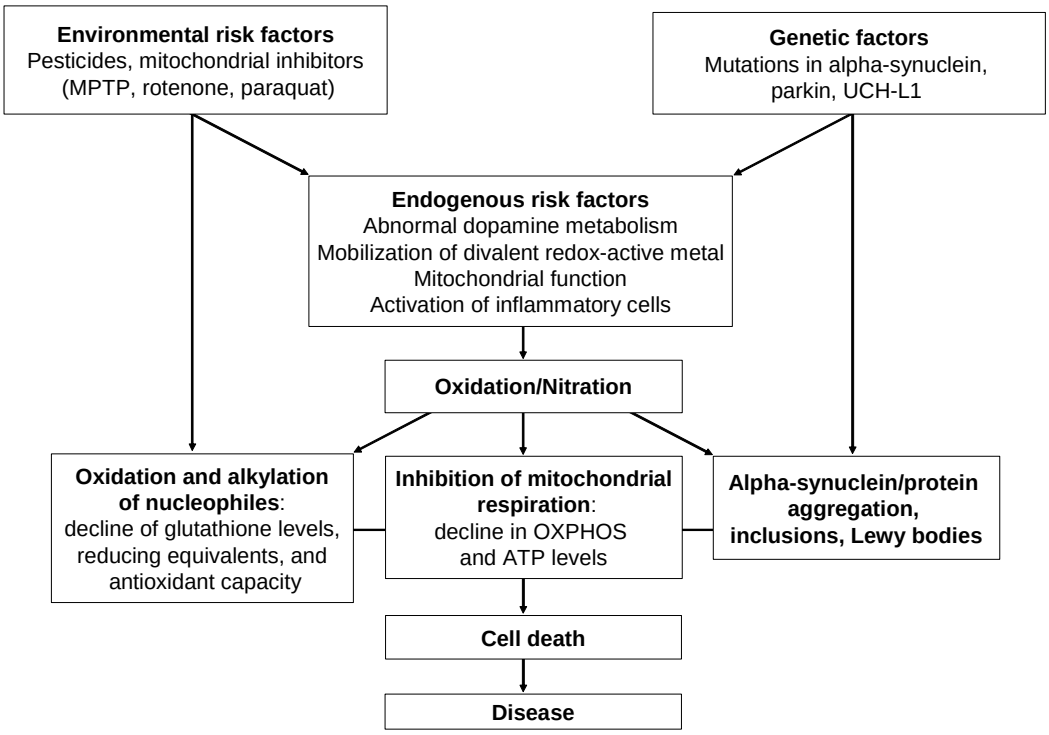
et al. 1998, El-Agnaf et al. 1998a), and these aggregates can induce apoptotic cell death (El-Agnaf et al. 1998b). Studies on transgenic mice overexpressing wild-type human α-synuclein have demonstrated progressive accumulation of α-synuclein and ubiquitin-immunoreactive inclusions in neurons in the neocortex, hippocampus and SN (Masliah et al. 2000). Aggregation of α-synuclein also appears to be stimulated with exogenous factors, such as the mitochondrial toxin rotenone, free radicals, and iron. Experiments in α-synuclein knockout mice have revealed that in the absence of α-synuclein function, these animals are resistant to MPTP-induced neurodegeneration (Dauer et al. 2002). There is some evidence that the neurotoxicity of α-synuclein might relate to its interaction with dopamine in dopaminergic neurons (Xu et al. 2002).

Cells use the ubiquitin-proteosomal pathway (UPP) in the degradation of proteins with abnormal conformation, and of proteins that are otherwise damaged or oxidized. Without degradation, these proteins tend to aggregate as inclusions. α-synuclein is also degraded by this pathway (Bennett et al. 1999, McLean et al. 2001, Rideaut et al. 2001). PD-causing mutations in α-synuclein results in a slightly slower degradation rate (Bennett et al. 1999). Lewy bodies also contain lipids, ubiquitin and UPP-related enzymes (Gai et al. 2000). UPP is implicated in AR-JP caused by mutations in the parkin gene (Tanaka et al. 2001). Parkin is involved in UPP by functioning as an ubiquitin E3 ligase and its functional loss in patients with parkin mutations is the molecular basis of AR-JP (Shimura et al. 2001). A glycosylated form of α-synuclein is one of the substrates of parkin and has been found to accumulate in the brains of patients with ARPD (Shimura et al. 2001). These findings provide a biochemical link between parkin and α-synuclein and it has been suggested that proteins contained within Lewy bodies might be targets of parkin-mediated ubiquitination (Chung et al. 2001a). UCH-L1 is the other main component in Lewy bodies and it also has an important role in the UPP. It belongs to a family of enzymes that is responsible for degrading polyubiquitin chains back to the ubiquitin monomer (Larsen et al. 1998). Thus interestingly, three proteins (α-synuclein, parkin and UCH-L1) encoded by PD-causative genes are either components of Lewy bodies and/or involved in the UPP. Consequently, it has been hypothesized that the UPP is a common underlying mechanism for the development of PD (Chung et al. 2001b, Krüger et al. 2002).

2.3.2. Oxidative stress and complex I

Over the past decade, several findings have suggested that oxidative stress plays a role in the pathogenesis of PD (Mouradian 2002, Ischiropoulos and Beckman 2003). Dopamine metabolism produces reactive species, and several indices of oxidative stress are observed in the SN, including elevation of iron, ferritin and nitric oxide. On the other hand, the activity of complex I of the mitochondrial respiratory chain is decreased in PD patients (Parker et al. 1989, Schapira et al. 1990, Shoffner et al. 1991). MPTP produces an acute parkinsonian syndrome that is indistinguishable from idiopathic PD. An metabolite of MPTP, MPP+, is the actual substance which inhibits complex I activity and is toxic to nigral cells (Nicklas and Heikkilä 1985). In a recent experimental study in rats, systemic partial inhibition of complex I was caused by the lipophilic pesticide rotenone. The exposure caused features of PD and a highly selective neurodegeneration of the nigrostriatal dopaminergic system, suggesting that these neurons have an intrinsic sensitivity to complex I defects (Betarbet et al. 2000). Further, an in vitro study indicated that complex I inhibition by rotenone induces accumulation and aggregation of α-synuclein and ubiquitin, progressive oxidative damage, and caspase-dependent death (Sherer et al. 2002). Interestingly, these results provide a link between mitochondrial dysfunction and α-synuclein. The fact that many other naturally occurring compounds and synthetic pesticides are potent inhibitors of complex I increases the importance of these findings (Degli et al. 1998). On the basis of current knowledge it is suggested that impaired OXPHOS seems to be central but not an initiating event for the development of PD (Figure 2) (Ischiropoulos and Beckman, 2003). A possible link between environmental and genetic factors might be that environmental factors causing increased oxidative stress induce the aggregation of α-synuclein in a genetically susceptible individual, leading to cell death (Mouradian 2002).

Figure 2. Proposed model of the pathogenesis of PD (adapted from Ischiropoulos and Beckman, 2003).



Abbreviations: ATP, adenosinetriphosphate; MPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OXPHOS, oxidative phosphorylation.

AIMS OF THE STUDY

Despite the extensive research that has been carried out, much controversy still exists regarding the role of genetics in the etiology of idiopathic PD. The causative genes for familial PD in the Finnish population are unknown. The involvement of complex I in the pathogenesis of PD is well established, but the role of single mutations in the MTND genes is unclear. This study was designed to assess the contribution of genetic factors in PD in the Finnish population using the family history method and complex segregation analysis (CSA). Molecular genetic methods were used to find causative mutations for familial PD and, in combination with mathematical methods, assess the effects of mtDNA sequence variation in the susceptibility to sporadic PD.

The specific aims of the study were:

1) To investigate the familial aggregation of PD in the Finnish population in a case-control study which included all identified patients in the province of Northern Ostrobothnia and controls from the same province (Study I).

2) To performe segregation analysis to test the models of a major dominant, additive, recessive, polygenic and sporadic inheritance in the total data set and in families with apparently familial PD. To examine the genetic heterogeneity between the EOPD and the LOPD (Study II).

3) To determine whether mutations in the genes encoding α-synuclein or parkin could be detected in Finnish patients with familial PD (Study III).

4) To determine the nonsynonymous to synonymous substitution ratio (K_a/K_s) of the seven MTND genes from Finns and whether differences in the K_a/K_s ratio in various mtDNA lineages contribute to the susceptibility to sporadic PD or clinical features (Study IV).

4. SUBJECTS AND METHODS

4.1. Subjects

4.1.1. Studies I-II

The familial aggregation study and complex segregation analysis (studies I-II) were conducted in the Department of Neurology, Oulu University Hospital (OUH), Finland during the years 1996-2000. Patients were selected from the population of Northern Ostrobothnia with a population of 358 411 on the prevalence date 31 December 1996. The register of patients with newly diagnosed PD has been maintained at the Department of Neurology in OUH since 1981 and represents the incidence of the disease in the population. This register was used to ascertain patients for this study. The corroboration of diagnosis was made based on chart review. Patients fulfilled the UK Parkinson's Disease Society brain bank clinical diagnostic criteria for PD (Daniel and Lees 1993). Patients with secondary parkinsonism or atypical clinical features, such as dementia at early stages of the disease, pyramidal signs, cerebellar signs, gaze paresis or significant focal lesions on CT or MRI scans were excluded. The control subjects were ascertained from the Central Population Registry of Finland. They were a random sample of individuals who were born before 1950 and did not have PD. The study was conducted with the permission of the ethics committee of the Medical Faculty, University of Oulu. Permission for chart review was obtained from the Finnish Ministry for Social Affairs and Health.

PD was diagnosed in 328 patients who were included in the familial aggregation study (study I). Family history data were obtained for 268 patients with PD and 210 controls. Demographic data of these subjects are presented in Table 3. The mean age of males (74.2±14.7 years) and females (77.2±7.8 years) who were not interviewed was higher than the mean age of interviewed males (68.9±9.4 years; $p=0.02$) and females (72.0±9.5 years; $p=0.001$). The controls were younger than the patients ($p<0.001$ for both sexes), but the mean age of the parents and siblings of the controls (60.6 ± 18.4 years) was not significantly different ($p=0.14$) from that of those of the patients (61.7±19.9 years).

The sample in the CSA (study II) consisted of nuclear families (probands and their siblings and parents) of 265 patients who participated in the familial aggregation study

(Study I). Individuals younger than 40 years of age were excluded from the analyses since the prevalence rates of PD for these individuals have not been estimated. Relatives of unknown age were also excluded from the analysis because their prior probability of affection was not known.

4.1.2. Studies III-IV

Subjects from Northern Savo, Finland were recruited in the molecular genetic studies (Studies III-IV) in the Department of Neurology, Kuopio University Hospital (KUH), during the years 1997-1998. Patients with parkinsonism were seen consecutively for two years in the out-patient clinic of the Department of Neurology, KUH. The UK Parkinson's Disease Society brain bank criteria were employed for the diagnosis of PD (Daniel and Lees 1993). Altogether 308 patients underwent an interview and neurological examination by the author. The following instruments were used for neurological evaluation of PD patients: Hoehn and Yahr stage, Unified Parkinson's Disease Rating Scale (UPDRS), Mini Mental State Examination (MMSE) and Clinical Dementia Rating (CDR). In total, PD was diagnosed in 235 patients and Parkinson's disease with dementia (PDD) in 34 patients. Of PD patients 215 had sporadic disease and 20 had affected first-degree relatives. Of PDD patients 30 had sporadic disease and 4 had familial disease. Except for four patients with PD and one patient with PDD, all these patients gave informed consent and blood samples were taken. Of the remaining patients evaluated, 19 had parkinsonism caused by neuroleptic medication or cerebrovascular events, 4 had DLB, 1 had frontotemporal dementia, 9 had isolated tremor, two had progressive supranuclear paresis and 3 had multisystem atrophy. These cases were excluded from the study.

During the years 1998-2000, the molecular genetic analysis of the α-synuclein gene and the parkin gene (Study III) was carried out in the Departments of Neurology and the DNA and Chromosome Laboratory, KUH. For this analysis, 22 patients with familial PD were ascertained through two sources (Table 3). First, sources collected during the familial aggregation study were used to include patients from Northern Ostrobothnia (Study I). Secondly, sources from the case series collected in KUH were used to include patients from Northern Savo. Altogether 50 relatives (40% male) were affected in 22 families participating in study III (Table 4). Confirmation of the diagnosis

of PD was based on chart review of all these cases. The segregation ratio for siblings was 0.27 (43 of 161) and for parents 0.05 (2 of 44). The mean age at onset was 62.3±10.2 years in the whole sample of familial patients (n=50). The mean age at onset was 57.0±10.9 years in 21 patients belonging to families with EOPD (<55 years), whereas the mean age at onset was 66.2±7.7 years in the families with LOPD. Eight of 50 patients were deceased and the mean age at death was 75.2 years (SD, 9.1; range, 57.9-83.6). Results of neuropathological examination were available for one patient showing neuronal and pigmentary loss, Lewy bodies in the SN and positive staining for α-synuclein in Lewy bodies consistent with definite PD. The mean duration of PD in 42 living patients was 7.5±4.6 years. The initial symptom was tremor in 82 percent of the patients and rigidity in 18 percent. Clinical examination showed tremor in 94 percent of the patients. Information concerning patient's response to dopaminergic drugs was available for 80 percent of patients and all these cases had sustained symptomatic benefit from these drugs.

In further analysis, 45 patients with sporadic PD were included (Table 3). These patients were also ascertained from the case series of KUH and met the same diagnostic criteria of idiopathic PD as familial patients, but had no family history of PD. The mean age at onset of PD in sporadic patients was 62.5±9.4 years. The mean duration of PD in these patients was 7.0±4.7 years. Tremor was the initial symptom in 71 percent of sporadic patients and it was observed in the clinical examination in 93 percent of these patients. The response to dopaminergic drugs was good in 89 percent of sporadic patients.

Sixty-seven control subjects were individuals without neurodegenerative diseases from the population of Eastern Finland (Table 3). Permission for the study was obtained from the ethics committee of KUH. All subjects provided informed consent prior to their inclusion in the study.

For the molecular genetic analysis of mtDNA polymorphisms (Study IV), 240 patients with sporadic PD were recruited from Northern Savo. These patients were consecutively selected among the outpatients attending the Department of Neurology, KUH. Controls included 107 individuals from Northern Savo. Controls were clinically examined and had no signs of neurodegenerative disorders. Determination of mtDNA

haplogroup was inconclusive in two patients with PD and in three controls. Therefore, the final number of subjects analyzed was 238 patients and 104 controls (Table 3).

Table 3. Demographic characteristics of PD patients and controls of individual studies.

Study	Group	N (% male)	Mean age±SD (y)
Studies I-II	PD patients	268 (50)	68.9±9.4 (male)
			72.0±9.5 (female)
	Controls	210 (49)	61.6±9.6 (male)
			67.1±10.9 (female)
Study III	FPD patients	22 (36)	73.5±10.4
	SPD patients	45 (40)	69.4±8.9
	Controls	67 (45)	72.7±5.2
Study IV	PD patients	210 (51)	69.0±9.3
	PDD patients	28 (70)	72.2±8.4
	Controls	104 (30)	71.9±6.2

Abbreviations: fPD, familial PD; sPD, sporadic PD.

Table 4. Twenty-two families with Parkinson's disease.

Family	Proband	Affected 1. degree relatives	Affected 2. and 3. degree relatives	Range of AAO (years)
NS-1	female	brother		56-75
NS-2	female	brother		60-63
NS-3	female	brother		53-65
NS-4	male	sister		68–70
NS-5	female	sister		60–69
NS-6	female	sister		63–84
NS-7	female	daughter		57–64
NS-8	male	brother		48–59
NS-9	female	brother	2 cousins	51–78
NS-10	female	father		52–78
NO-1	female	sister		58–61
NO-2	female	sister		67–74
NO-3	male	brother		64–65
NO-4	male	sister		58–58
NO-5	female	daughter		58–72
NO-6	male	sister		55–72
NO-7	female	2 brothers		46–58
NO-8	male	2 brothers	Sister's daughter	42–74
NO-9	female	sister		71–78
NO-10	female	son		40–61
NO-11	male	sister		67–80
NO-12	male	sister		45–60

Abbreviations: NS, family from Northern Savo; NO, family from Northern Ostrobothnia.

4.2. Methods

4.2.1. Familial aggregation study (Study I)

The patients with PD and the controls were submitted to a detailed interview designed to obtain family history information. The interview was made by telephone and the probands were informed about the investigation in a letter one to two weeks before the interview. If the proband was unable to provide the information due to dementia or other reason then a next of kin was interviewed. The following data were recorded from all first-degree relatives: sex, the year of birth, year of death in case of deceased subjects, and the history of PD, tremor or movement disorders. The place of birth of the parents was also elicited. If the proband ascertained one of these symptoms or disorders in a family member, corroboration of diagnosis was made on the basis of the medical records.

A positive family history was defined as the presence of PD in at least one first degree relative. The diagnosis of PD was considered to be definite if the review of the medical records confirmed the diagnosis. The medical records were not available in all ascertained affected cases and in those cases the diagnosis of PD was based only on the information from the proband. All interviews and chart reviews were performed by the same person, as multiple interviewers might have caused diagnostic variability in the sample.

4.2.2. Complex segregation analysis (CSA)

Model Complex segregation analysis (CSA) is based on the distribution of the disease in nuclear families (parents and their offspring). Family data from familial aggregation study (Study I) were used in CSA based on the unified mixed model as implemented by POINTER (Lalouel and Morton 1981). The computer program, POINTER, is available at ftp://cedar.genetics.soton.ac.uk/pub/PROGRAMS/pointer/. The model assumes that the liability to the disease can be described by an underlying continuous liability scale in which a major locus (g), a polygenic component (c), and environmental effects (e) operate independently, the liability being defined as $x=g+c+e$. The respective variances of these parameters are denoted as $V=G+C+E$. The relative contribution of the polygenic component is defined by H, the heritability, which reflects genetic transmission not ascribed to a major gene or cultural transmission: $H=C/V$. The

major locus has two alleles, and the genotype frequencies follow the Hardy-Weinberg equilibrium. Three parameters define the major locus: *q*, the frequency of the disease allele; *t,* the distance measured in standard deviations on the liability scale between the two homozygous genotypes; and *d,* the degree of dominance. If *d*=0, the abnormal gene is recessive, whereas if *d*=1, it is dominant, and when *d*=0.5 the effect of the abnormal allele is additive.

Liability classes The phenotypes were defined here as dichotomies of affection status: normal versus affected. Thus, the liability to affection was represented by *x*, affection being defined by a threshold on the liability scale; affection occurs when *x* is greater than a given threshold. Individuals were assigned to nine liability classes (Table 4) according to their prior probability of affection based on the age- and sex-specific prevalence rates for PD in Finland (Kuopio et al., 1999). Age was taken to be age at the time of ascertainment or at death. There was only one proband in each family, and therefore the ascertainment probability 0.001 was used in the analysis, corresponding to single selection.

Table 4. Age and sex-specific prevalences of PD in Finland based on data by Kuopio et al. (1999). The numbers in parentheses refers to the liability class as defined for POINTER.

Age (years)	Women	Men
40-49	0.0005 (1)	0.0005 (1)
50-59	0.0015 (2)	0.002 (3)
60-69	0.004 (4)	0.005 (5)
70-79	0.0095 (6)	0.017 (7)
80+	0.0075 (8)	0.0165 (9)

CSA The testing of genetic hypotheses proceeds by keeping the relevant parameters from *d*, *t*, *q*, and *H* constant, whereas the remaining parameters are estimated by maximizing the likelihood of the phenotypes in the families. Conditional likelihoods were used. The difference between the minus twice the log likelihood plus a constant (-

2lnL+*k*) calculated under a general model (with *m* parameters) and under a reduced model (with *n* parameters) is asymptotically distributed as χ^2 with *m*-*n* degrees of freedom. Alternatively, the hypotheses can be compared directly by using the Akaike information criterion (AIC; Akaike 1974), which is -2lnL+*k* plus twice the number of free parameters in the model. The model with the lowest AIC is taken to give the best fit to the data. Comparison by means of AIC values has the advantage that one model does not have to be a subset of the other.

Test of genetic heterogeneity The data set consisting of all nuclear families was analyzed first in order to determine whether polygenic or major locus models would explain the occurrence of PD entirely. The data were subsequently divided into families with EOPD (probands AAO<55 y) and LOPD to determine whether the genetic background to PD was different in appearance in these groups. Parameters for the polygenic, dominant, and recessive models were estimated separately in these two groups. The difference between the summed likelihoods in the partitioned analysis and the likelihood of the total data set is asymptotically distributed as χ^2 with $p(g-1)$ degrees of freedom, where *p* is the number of iterated parameters and *g* is the number of subgroups.

CSA in the subgroup of familial PD Finally, the families with at least one case of PD among the parents or siblings in addition to the proband were analyzed separately, as choosing probands with affected family members results in an enrichment of disease alleles in the sample (Wright et al. 1999). POINTER handles the selection of familial cases in the following way. If the additional case is a sibling, an approximate sampling correction consists of defining the proband as a pointer, whereas his siblings are treated under truncate selection. In other instances, conditioning on parents or pointers accounts for such a mode of selection (Morton et al. 1983).

4.2.3. Molecular genetic analysis of the *α*-synuclein and the parkin gene (study III)

Sequence analysis of the α-synuclein gene DNA was extracted from peripherial blood lymphocytes using a standard phenol-chloroform extraction method (Vandenplas et al. 1984) and subjected to analysis by polymerase chain reaction (PCR). The non-coding (exon 1'; Genebank/EMBL accession number U46896 sequence 2701-3078) and coding (exons 2-6; Genebank/EMBL accession numbers U46897-46901) exons of the

α-synuclein gene were PCR amplified from genomic DNA with the primer sequences indicated in the Table 5.

The PCR products were purified using a QIAquick PCR purification kit (Qiagen) and cycle-sequenced in both directions with the ABI PRISM 310 genetic analyser (Applied Biosystems, CA, USA) and Sequencing Analysis Program 3.7 (Applied Biosystems) by utilizing the dR fluorensence terminator kit (Applied Biosystems) with Taq Polymerase (Promega). All exons of the α-synuclein gene were analysed from patients with familial PD. Because three novel exon-1' non-coding alterations were found in the α-synuclein gene, a part of exon 1' comprising the nucleotide sequence $T_{10}A_7$ (U46896; sequence 3014-3030) was also analysed from controls and patients with sporadic PD using direct PCR sequencing.

Table 5. Primer sequences and PCR product size of the α-synuclein gene (Vaughan et al. 1998).

α-synuclein exon*	Primers 5'/3', forward and reverse	Product size (bp)
Exon 1' (Exons 1 and 2)	GAGAAGGAGGAGGACTAGGAGG CGGCGTTCTCCAGGATTTC	499
Exon 2 (Exon 3)	GTCTCACACTTTGGAGGGTTTC	395
Exon 3 (Exon 4)	CACCTACCTACACATACCCTCTGACTC GCTAATCAGCAATTTAAGGCTAG	215
Exon 4 (Exon 5)	GATATGTTCTTAGATGCTCAG CGATGGCTAGTGGAAGTGG	325
Exon 5 (Exon 6)	CGATGGCTAGTGGAAGTGG CGGAGGCATTGTGGAGTTTAG	373
Exon 6 (Exon 7)	CCACGTAATGAGCATGTAGAGAGC GACTGGGCACATTGGAACTGAG	189
	GCTGTCAGTGCTGATGCGTAATTG	

* Exon numbering used in the study of Vaughan et al. 1998 is indicated in parentheses.

Mutation analysis of the parkin gene Eight point mutations in the parkin gene described by Abbas et al. (1999) were searched from patients with familial PD. The mutation was characterized either directly using the ABI PRISM 310 genetic analyzer or through restriction enzyme digestion and gel electrophoresis of the products. Mutations Gln34/Stop37, Asn52/Stop81, Arg275Trp and Trp453Stop were analyzed as described by Abbas et al. (1999) (Table 6). For Trp74/Stop81, a two nucleotide insertion at exon 3 of the parkin gene, oligo 5'-ACCTGGATCAGC AGAGCATT-3' was fluoroamid-labelled and used as a forward primer, and oligo 5'-TCCTTCCTGCTGTCAGTGTG-3' was used as a reverse primer. After PCR the amplified products were analysed using an ABI PRISM 310 genetic analyser.

For Lys161Asn, Arg256Cys and Thr415Asn mismatched primers were designed to create a PCR-restricted fragment length polymorphism (RFLP) method for mutation detection (Table 6). PCR amplification was performed in all reactions with Ampli*Taq*Gold polymerase (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations.

The cycling conditions for all reactions were denaturation at 96 °C for 10 min following 35 cycles of 94 °C for 1 min, 50-58 °C for 1 min and 72 °C for 1 min. Primer extension was carried out at 72 °C for 6 min. PCR products were digested for 3 h in a 25-µl incubation volume using 5 units of an appropriate restriction enzyme as recommended by the manufacturer, after which cleavage electrophoresis was carried out on 1.5-3% agarose gel. The gel was stained with ethidium bromide and photographed.

Table 6. Primer sequences, restriction enzymes and PCR product size of the parkin gene mutations.

Mutation	Primers 5'/3', forward and reverse	Restriction enzyme	Product size (bp)
Gln34/Stop37	CAAAGAGTGCAGCCGGGATA	-	wt:308
Asn52/Stop81	GGGTCAAGGTGAGCGTTG ATGTTGCTATCACCATTTAAGGG	*Fok*I	m:306 wt:278+30
Arg275Trp	AGATTGGCAGCGCAGGCGGCATG TGCCTTTCCACACTGACAGGTACT	*Sau*3AI	m:222+57+30 wt:142+97
Trp453Stop	TCTGTTCTTCATTAGCATTAGAGA GTTTGGGAATGCGTGTTTT	*Nla*IV	m:239 wt:142+17+35+61
Trp74/Stop81	AGAATTAGAAAATGAAGGTAGACA ACCTGGATCAGCAGAGCATT	-	m:159+35+61 wt:210
Lys161Asn	TCCTTCCTGCTGTCAGTGTG CAAAGAGTGCAGCCGGGATA	E*co*RV[a]	m:212 wt:70
Arg256Cys	GGGTCAAGGTGAGCGTTG TGCCTTTCCACACTGACAGGTACT	*Fau*I[a]	m:51+19 wt:145+94
Thr415Asn	TCTGTTCTTCATTAGCATTAGAGA TCCAAAGAAACCATCAAGAAAAGTA	M*ae*III[b]	m:239 wt:170
	AGGCACCTTCAGACAGCATC		m:148+22

Abbreviations: wt, Wild type; m, mutation. [a]New England Biolabs, Beverly, MA, USA. [b]Roche Molecular Biochemicals, Indianapolis, IN, USA.

4.2.4. Analysis of sequence variation in MTND genes (Study IV)

DNA analyses Total DNA was isolated from blood cells using a QIAamp Blood Kit (Qiagen, Hilden, Germany). Mitochondrial DNA haplogroups were determined by restriction fragment analysis of polymorphic sites described in the Table 7 (Torroni et al. 1996, Finnilä et al. 2001a). Each mtDNA was defined to one of the nine European haplogroups (H, I, J, K, T, U, V, W, X) and for subsequent analyses haplogroup clusters HV, KU, JT and IWX were used. Determination of haplogroup was inconclusive in two patients with PD and three controls, and they were excluded from the analyses.

Table 7. Restriction enzymes used for detection of mtDNA haplogroups (Finnilä et al. 2001a).

Haplogroup	Polymorphic sites
H	-7025*Alu*I, -10394*Dde*I
V	-4577N*la*III, -10394*Dde*I
U	+12305*Dde*I, -10394*Dde*I
K	+12305*Dde*I, +10394*Dde*I
T	+15606*Alu*I, -10394*Dde*I
J	-13704*Mva*I, +10394*Dde*I
I	-1715*Dde*I, +8251*Ava*II, +10394*Dde*I
W	+8251*Ava*II, -10394*Dde*I
X	-1715*Dde*I, -10394*Dde*I
Z	+10397*Alu*I, +10394*Dde*I

-, loss of restriction site; +, gain of restriction site. The number indicates the 5´ position of the recognition sequence. DdeI restriction site at position 12305 is created by use of a mismatched oligonucleotide primer in the polymerase chain reaction.

Diversity indices and neutrality tests The complete mtDNA sequence has been determined in 183 randomly selected Finnish mtDNAs representing the European haplogroups present in this population (Finnilä et al. 2001a). The MTND genes in these sequences were analyzed in terms of nucleotide polymorphism indices and phylogenetic tests of neutrality. The total number of nucleotide sites in the seven MTND genes was 6321 after the exclusion of the initiation and termination codons.

Nucleotide diversity indices (θ_π, θ_s and $\theta_{\eta s}$) and neutrality tests (D, F* and Fs) were calculated for the Finnish sequences. The methods used for estimating θ values included k or θ_π, the average number of pairwise nucleotide differences (Tajima 1983), Watterson's estimate (θ_s), based on the number of polymorphic sites in the sample (Watterson 1975), and $\theta_{\eta s}$, based on the number of singleton mutations (η_s) in the sample (Fu and Li 1993). Under neutrality and certain demographic assumptions (Nielsen 2001) these three estimates should yield similar values (Simonsen, Churchill and Aquadro 1995). However, θ_s and $\theta_{\eta s}$ are affected by the presence of low-frequency alleles in the sample, whereas these have little impact on θ_π, leading to differences between the estimates when selection is present (Fu and Li 1993). These differences form the basis of phylogenetic tests of neutrality, including Tajima's D, which is based on the difference between θ_π and θ_s and its variance (Tajima 1989), Fu and Li's F*, which is based on the difference between θ_π and $\theta_{\eta s}$ and its variance (Fu and Li 1993), and Fu's Fs, which is based on the probability of the observed number of haplotypes or more being observed under neutrality (Fu 1997). An excess of low-frequency alleles results in negative values for these tests, whereas a relative lack of low-frequency alleles results in a shift towards positive values. Negative values have been found for human mtDNA, indicating selection against slightly deleterious mutations, changes in population structure, or both (Gerber et al. 2001). A program package, dnastats, (available at http://cc.oulu.fi/~jukkamoi/mtres/) was used for the analyses.

Nonsynonymous/synonymous rate ratios in MTND genes In the present study an African sequence (NC_001807.4, GenBank accession number AF347015) was used as an outgroup representing the most recent common ancestor for human evolution. As in the following example (Figure 3), the numbers of nonsynonymous and synonymous substitutions and sites in the seven MTND genes were counted between the MRCA sequence and each of the 183 Finnish sequences (Nei and Gojobori 1986) by using

DnaSP. Initiation and termination codons were excluded. Nonsynonymous to synonymous substitution ratio (K_a/K_s) was calculated for each sequence. Distributions of K_a/K_s values between clusters were compared using the Wilcoxon and Kruskal-Wallis rank sum tests as implemented in R 1.4.1 (Ihaka and Gentleman 1996). The K_a/K_s ratio indicates the relative level of selection against nonsynonymous mutations and is insensitive to factors such as population history. K_a/K_s is smaller when the average selection against nonsynonymous substitutions is greater, and K_a/K_s exceeds unity when nonsynonymous mutations are favourable, i.e. adaptive.

Figure 3. Alignment of human and orangutan MTND1 gene 5´ end and the translation of the sequences into the aminoterminal end of ND1 subunit. Synonymous substitutions are underlined; nonsynonymous substitutions in bold. The nonsynonymous to synonymous substitution ratio (K_a/K_s) in this sequence is 5/9= 0.56

```
          M   P   M   T   N   L   L   L   L   I   V   P   I   L   I   A   M   A
human ATACCCATGACCAACCTCCTACTCCTCATTGTACCCATTCTAATCGCAATGGCA
ponpp ATGCCTGTAATCAACCTCCTGCTCCTCACTATATCTATCCTGATCGCCATAGCA
          M   P   V   I   N   L   L   L   L   T   M   S   I   L   I   A   M   A
```

Abbreviations: ponpp, Pongo pygmaeus pygmaeus (Bornean orangutan).

4.2.5. Statistical analysis

In the familial aggregation study, an independent sample *t* test and χ2 analysis was used to determine differences between patients and controls. Kaplan-Meier survival analysis and log rank statistics were used to compare age-specific cumulative incidence of PD between first degree relatives of patients and controls.

Frequencies of the polymorphic alleles or genotypes of the α-synuclein gene were analysed with the χ^2-test, Fisher's exact.

MtDNA haplogroup cluster frequencies between patients with PD, patients with PDD and controls were compared using the χ^2-test. Differences in age, age at onset, age at diagnosis, and disease duration between samples were tested using the Mann-Whitney test for two groups and the Kruskall-Wallis test for more than two groups. Univariate analyses of variance were performed to assess whether UPDRS total or motor scores differed between PD patient groups formed by the mtDNA haplogroup. In these analyses disease duration was a co-variate.

5. RESULTS

5.1. Familial aggregation of PD (Study I)

Ten percent of the patients reported PD among first degree relatives, whereas the corresponding frequency was 3.8% in the controls (p=0.01). Twenty-six of the probands reported an affected parent or sibling, and one female proband had an affected daughter, this family being excluded from further analyses. The 26 probands reported 29 affected siblings and parents. Review of the patient charts of the siblings disclosed 10 definite cases of PD, and the remaining 11 siblings and eight parents had a diagnosis of possible disease. The crude segregation ratio was 0.27 for the siblings and 0.17 for the parents. The controls reported eight affected parents and siblings, of whom three were definite patients with PD and five had possible disease. The frequency of definite or possible PD was thus 1.6% in first degree relatives of the patients and 0.6% in first degree relatives of the controls. The relative risk of PD among the first degree relatives of the patients was 2.9 (95% CI 1.3-6.4).

The data based on chart review showed that the clinical features of 26 familial and 242 sporadic patients were similar except for the age at onset, which was 63.7±8.7 years in patients with familial PD and 60.0±10.8 years in patients with sporadic PD (p=0.05). The age at death of the parents was not different between those families in whom affected members were found only among siblings and those families in whom affected members were present in two generations.

The age-specific cumulative incidence of PD in 476 parents and 1149 siblings of the patients (Figure 3) was significantly different from that in 394 parents and 907 siblings of the controls (p=0.009). The cumulative incidence of disease by the age 90 years was 0.10 for parents and siblings of the patients and 0.03 for parents and siblings of the controls.

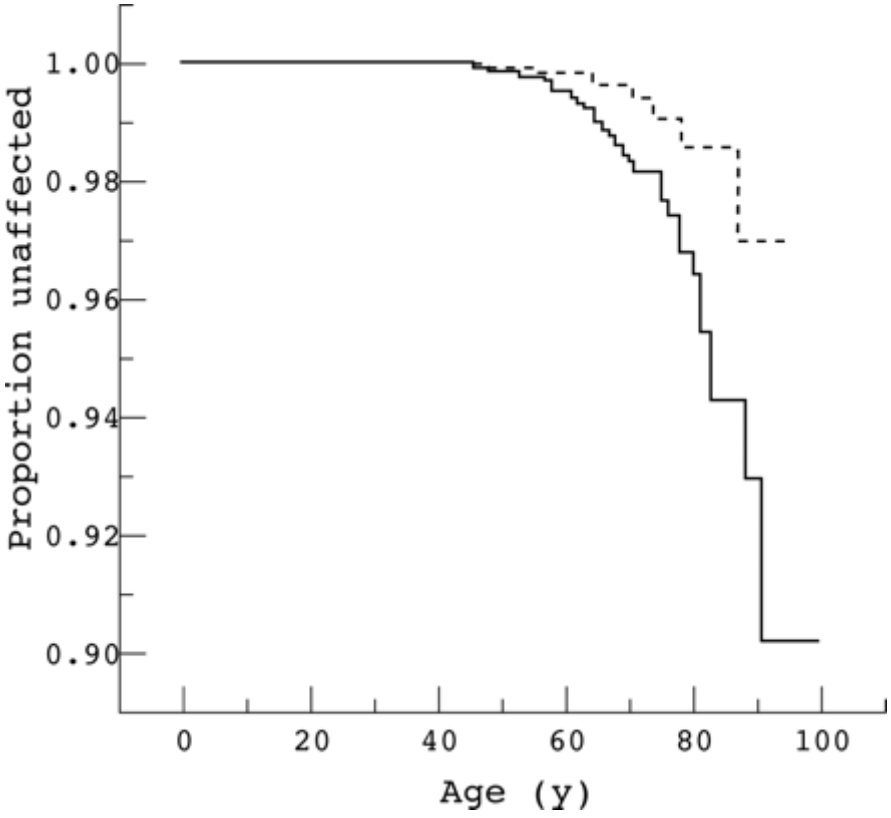


Figure 3. Proportion of first-degree relatives remaining unaffected with respect to PD. Solid line, relatives of patients with PD; dotted line, relatives of controls.

5.2. Complex segregation analysis (Study II)

The series consisted of 265 nuclear families with 1677 individuals. Five mothers and two fathers were affected, and a sibling was affected in 19 families. In the CSA performed on the total series, the sporadic model was strongly rejected (mixed vs. sporadic, χ^2=26.78, df=4, P<0.0001; Table 8). None of the three models that incorporate a major locus explained the observed segregation pattern better than the polygenic model. The estimated probability of affection at the age of 80 was 9%-16% for carriers of the dominant gene and 12%-20% for homozygotic carriers of the recessive gene. Comparison of AIC values indicated that the polygenic model gave the best fit to the data.

Table 8. Results of the CSA for PD among all families.

Model	d	t	q	H	-2lnL+k	AIC
Sporadic	-	-	(0)[a]	(0)	-2772.64	-2772.64
Polygenic	-	-	(0)	0.401	-2799.18	-2797.18
Dominant	(1)	1.28	0.024	(0)	-2798.64	-2794.64
Additive	(0.5)	2.07	0.049	(0)	-2799.32	-2795.32
Recessive	(0)	1.47	0.195	(0)	-2798.94	-2794.94
Mixed	0.52	2.02	0.052	0.009	-2799.42	-2791.42

Abbreviations: d, dominance; t, displacement; q, gene frequency; H, heritability; -2lnL, minus twice the log likelihood; k, a constant; AIC, Akaike information criterion.

The series was then divided according to the age of onset of PD in the proband. Heterogeneity tests between the families of probands with EOPD and LOPD revealed significant heterogeneity under the polygenic (χ^2=6.46, df=1, *P*=0.011), dominant (χ^2=7.17, df=2, *P*=0.028), and recessive (χ^2=7.07, df=2, *P*=0.029) models. Under the polygenic model, the estimate for common heritability not ascribed to a major gene (*H*) was higher in the late-onset families than in the early-onset families (Table 9). Under the dominant and recessive models, the estimated gene frequencies were much higher in the families with EOPD than in families with LOPD (Table 9).

Table 9. Heterogeneity tests for the families of probands with EOPD or LOPD.

Model	Polygenic		Dominant			Recessive		
	H	-2lnL+k	t	q	-2lnL+k	t	q	-2lnL+k
EOPD	0.169	-748.69	1.88	0.152	-749.02	1.82	0.509	-748.98
LOPD	0.453	-2056.95	1.48	0.011	-2056.79	1.75	0.123	-2057.03
χ^2	6.46		7.17			7.07		
df	1		2			2		

Abbreviations: H, heritability; -2lnL, minus twice the log likelihood; k, a constant; t, displacement; q, gene frequency; χ^2 , heterogeneity statistics; df, degrees of freedom.

The subset of familial cases, consisting of 26 families with 190 members, was then analyzed separately. There were no families with affected individuals among both siblings and parents. The proband was defined as a child's pointer, if the additional affected person was a sibling, and the segregation analysis was carried out with the likelihood conditional on parents and pointers. The sporadic and polygenic models were rejected (mixed vs. sporadic, χ^2=12.3, df=4, *P*=0.015; mixed vs. polygenic, χ^2=9.55, df=3, *P*=0.023), whereas the major locus models were not (mixed vs. recessive, χ^2=0.03, df=2, *P*=0.99; mixed vs. additive, χ^2=2.59, df=2, *P*=0.27; mixed vs. dominant, χ^2=1.94, df=2, *P*=0.38). The general major locus model with an unrestricted degree of dominance (*d*) converged to the recessive model (Table 10). Under the recessive model, the estimated probability of affection for homozygotic carriers was 0.50 for individuals 40-49 years of age and 0.82-0.89 for individuals over 80 years of age (Table 8). The proportion of affected individuals who were homozygotic carriers was estimated to be 0.20 among individuals 40-49 years of age, 0.067-0.085 among individuals 50-59 years of age, and 0.01-0.02 among individuals over 80 years of age. Both the probability of affection, given the genotype, and the estimated proportion of individuals with PD who were gene carriers were lower under the dominant model than under the recessive model (Table 11).

Table 10. Results of CSA for PD among families with more than one affected individual.

Model	d	t	q	H	-2lnL+k	AIC
Sporadic	-	-	(0)[a]	(0)	13.89	13.89
Polygenic	-	-	(0)	0.0036	11.14	13.16
Dominant	(1)	2.30	0.00014	(0)	3.53	7.53
Additive	(0.5)	2.39	0.026	(0)	4.18	8.18
Recessive	(0)	3.36	0.014	(0)	1.62	5.62
Mixed	0	3.39	1.014	0.0019	1.59	9.59

Table 11. Characteristics of the autosomal dominant and recessive major locus models that provided the best fit to data set consisting of families with more than one affected individual.

Liability class	Age (years)	Gender	Dominant			Recessive		
			P (affection|genotype) GG	GG' or G'G'	P(G'|affection)	P (affection|genotype) GG or GG'	G'G'	P(G'|affection)
1	40-49	Both	0.00046	0.15408	0.08827	0.00040	0.50264	0.20036
2	50-59	Women	0.00143	0.24661	0.04710	0.00137	0.64226	0.08534
3	50-59	Men	0.00192	0.27641	0.03959	0.00187	0.67704	0.06747
4	60-69	Women	0.00390	0.35812	0.02565	0.00385	0.75633	0.03769
5	60-69	Men	0.00489	0.38735	0.02219	0.00485	0.78019	0.03110
6	70-79	Women	0.00937	0.47863	0.01443	0.00933	0.84313	0.01769
7	70-79	Men	0.01684	0.56884	0.00959	0.01683	0.89163	0.01045
8	80+	Women	0.00737	0.44385	0.01695	0.00734	0.82101	0.02182
9	80+	Men	0.01634	0.56409	0.00979	0.01633	0.88936	0.01074

(G wild-type, G'mutant allele)

5.3. Molecular genetic analysis of the α-synuclein and the parkin gene (Study III)

Twenty-two unrelated patients with familial PD did not harbour mutations or polymorphisms in the coding exons 2-6 of the α-synuclein gene. In addition, consensus sequences at the exon-intron splice junctions were found to be intact. Sequencing of the non-coding exon 1' of the α-synuclein gene, however, revealed three novel alterations in the $T_{10}A_7$ sequence of exon 1', including insertion of A ($T_{10}A_8$). Two of these alterations encompassed of replacement of A by T ($T_{11}A_6$) and AA by TT ($T_{12}A_5$). To assess further the role of these alterations, exon 1' of the α-synuclein gene was sequenced from 67 control subjects. The sequence analysis showed that these alterations were also present in the control subjects (Table 12). The frequencies of the exon 1' polymorphic genotypes or alleles between familial PD patients and control subjects revealed no statistically significant differences (Fisher's exact test, p = 0.08 and 0.30, respectively). Individual allele analysis showed borderline association of $T_{10}A_8$ allele with familial PD (Pearson's χ^2 test, p=0.05; OR= 3.0; 95% CI, 1.0 to 9.4).

By including 45 patients with sporadic PD in the analysis, no statistically significant differences in the genotype and allele distribution of exon 1' alterations were observed between PD and control subjects (Fisher's exact test, p = 0.23 and 0.22, respectively). No significant association for sporadic PD was observed in the individual allele analysis of $T_{10}A_8$ allele.

No alterations were found in the selected mutation analysis of the parkin gene.

Table 12. Genotype and allele frequencies of the exon 1' polymorphisms of the α-synuclein gene in familial PD

Subjects		Genotype Frequency								
	N	$T_{10}A_7$/ $T_{10}A_7$	$T_{10}A_7$/ $T_{12}A_5$	$T_{10}A_7$/ $T_{11}A_6$	$T_{10}A_7$/ $T_{10}A_8$	$T_{12}A_5$/ $T_{12}A_5$	$T_{12}A_5$/ $T_{11}A_6$	$T_{12}A_5$/ $T_{10}A_8$	$T_{11}A_6$/ $T_{10}A_8$	$T_{10}A_8$/ $T_{10}A_8$
PD	22	0.41	0.14	0.05	0.23	0.05	0.05	0.00	0.09	0.00
Controls	67	0.45	0.27	0.12	0.10	0.03	0.00	0.02	0.00	0.02
		Allele Frequency								
		$T_{10}A_7$	$T_{12}A_5$	$T_{11}A_6$	$T_{10}A_8$					
PD	44	0.61	0.14	0.09	0.16					
Controls	134	0.70	0.16	0.06	0.08					

N, number of genotypes and alleles in familial PD patients and controls. PD and control genotypes are in Hardy-Weinberg equilibrium. Fisher's exact test for genotype frequencies: PD/C, p=0.08. Fisher's exact test for allele frequencies: PD/C, p=0.30.

5.4. Variation in MTND genes and risk of PD (Study IV)

Nucleotide diversity indices and neutrality tests in MTND genes The complete mtDNA sequence has been determined in 183 randomly selected Finnish mtDNAs belonging to the European haplogroups (Finnilä et al. 2001a). The MTND genes in these sequences were analyzed in terms of nucleotide diversity indices and phylogenetic tests of neutrality. Nucleotide diversity indices θ_π, θ_s, $\theta_{\eta s}$ showed that cluster HV differed from the remaining clusters in showing an increased frequency of singleton mutations (Table 13). In agreement with the analyses on nucleotide diversity, neutrality tests (D, F* and Fs) yielded the most negative values for cluster HV.

The MTND genes were then analyzed in terms of the average number of nonsynonymous differences and were found to harbour 136 transitions and 7 tranversions (Table 13). The haplogroup clusters differed in the K_a/K_s ratio, clusters HV and KU having a lower ratio than clusters JT and IWX. K_a/K_s distributions differed between the clusters ($p<0.0001$, Kruskal-Wallis test), and the differences suggested a relative excess of nonsynonymous mutations in clusters JT and IWX. For subsequent analyses, the clusters HV and KU with a low K_a/K_s ratio were combined into a supercluster HVKU with K_a/K_s of 2.436. Similarly, clusters JT and IWX with a higher K_a/K_s ratio were combined into supercluster JTIWX with K_a/K_s of 4.268. The location of the K_a/K_s distribution was estimated to differ by 0.08 ($p<0.00001$, Wilcoxon rank sum test).

Table 13. Diversity indices and neutrality tests in the MTND genes in Finnish mtDNA sequences (excluding haplogroup Z).

Haplo group	N	Haplotype diversity±SE	Transitions	Transversions	K_a/K_s±SD	S	η	η_s	θ_s±SE	π±SE	$\theta_{\eta s}$	D	F*	Fs
HV	58	0.832±0.036	28	1	2.569±0.096	29	29	20	6.265±2.007	2.766±1.648	19.655	-1.8	-3.9	-7.2
KU	43	0.933±0.020	44	4	2.256±0.061	47	48	10	10.863±3.423	7.114±3.780	9.767	-1.2	-0.35	-2.6
JT	28	0.966±0.017	36	1	4.000±0.133	37	37	11	9.508±3.300	6.937±3.743	10.607	-1.0	-0.54	-4.9
IWX	54	0.848±0.035	28	1	4.407±0.159	29	29	8	6.364±2.058	7.272±3.837	7.852	0.48	-0.13	0.75

Abbreviations: N, number of sequences; S, number of segregating sites; η, total (minimum) number of mutations; η_s, number of singleton mutations; k, average number of pairwise differences; π, nucleotide diversity; SE, standard error; D, Tajima's D; F*, Fu and Li's test; Fs, Fu's Fs.

Table 14. Frequencies of mtDNA haplogroup clusters in controls, PD patients and PDD patients.

Haplogroup cluster	Controls		PD		PDD	
	N	%	N	%	N	%
HV	59	57	120	57	10	36
KU	33	32	56	27	12	43
JT	5	5	19	9	1	4
IWX	7	7	15	7	5	18
HVKU	92	89	176	84	22	79
JTIWX	12	12	34	16	6	21

Nonsynonymous to synonymous substitution rate in MTND genes and risk of PD There were no differences in haplogroup frequencies between the sexes. The frequencies of mtDNA haplogroup clusters were similar among PD patients and controls (Table 14), whereas the frequencies in PDD patients differed from those in PD patients and the controls (p=0.035).

Supercluster JTIWX was found to be more frequent among PD patients and even higher among patients with PDD (Table 14). The patients belonging to supercluster JTIWX had a slightly higher risk for PD (OR 1.48, 95% CI 0.73-3.00; P = 0.27) and PDD (OR 2.09, 95% CI 0.71-6.19; P = 0.18) compared with those belonging to supercluster HVKU. The clinical features of patients with PD or PDD were then compared between the two superclusters. No differences were observed between patients in these two groups in age at onset (p=0.73 for PD; p=0.26 for PDD,), duration of PD (p=0.15 for PD; 0.33 for PDD), total UPDRS score (p=0.12 for PD; p=0.55 for PDD) or motor UPDRS score (p=0.24 for PD; p=0.69 for PDD).

The effect of the 10398A>G polymorphism on risk of PD or PDD and clinical features Finally, the effect of the 10398A>G polymorphism was analyzed by comparing the risk of PD or PDD and clinical features between carriers (haplogroup I, J or K) and non-carriers (haplogroups H, V, U, T, W or X) of 10398A>G. The subjects belonging to haplogroups IJK had a slightly decreased risk of PD (OR 0.76, 95% CI 0.36-1.6; P = 0.49), but increased risk of PDD (OR 2.09, 95% CI 0.71-6.19; P = 0.18) compared with those belonging to other haplogroups. The mean age at onset of PDD

was lower among patients belonging to haplogroup IJK compared with that among patients belonging to other haplogroups (56.8±9.4 y vs. 66.3±10.5 y; P = 0.05). The age at onset did not differ in patients with PD between haplogroup IJK and other haplogroups (60.7±10.0 y vs. 62.6±10.2 y; P = 0.38).

6. DISCUSSION

6.1. Methodological considerations

6.1.1. Familial aggregation studies

There have been many case-control studies on familial aggregation of PD. These studies pose a number of methodological problems concerning the study populations, design, case ascertainment, collection of family history information and case verification. In several studies probands were selected from consecutive cases of movement disorder clinics and the results may be affected by sampling bias (Morano et al. 1994, Payami et al. 1994, Bonifati et al. 1995, Taylor et al. 1999, Preux et al. 2000). There are four population-based studies providing valid estimates of the odds ratios for PD in relatives (Semchuk et al. 1993, Marder et al. 1996, Elbaz et al. 1999, Kuopio et al. 2001).

In the present study, controls were ascertained randomly from the population and patients were identified from a register of patients with newly diagnosed PD which is maintained in the Department of Neurology, OUH. This register provides a good representation of the incident cases of PD since OUH serves as a primary site of treatment for these patients in the province of Northern Ostrobothnia. A great majority of patient with PD in this area will at some point be referred to OUH for diagnosis or treatment since they need confirmation of their disease by a neurologist to get the cost of medications completely refunded by the National Health Insurance. Therefore, we believe that the potential sampling effect is avoided in our study and it provides a population-based estimate of familial aggregation of PD.

The Finnish population offers many advantages in a study of the genetic epidemiology of disease. The population is homogenous in terms of cultural and environmental factors as well as genetic background. This issue is much more complicated in the US population, where individuals come from different ethnic backgrounds. In the case-control study by Marder et al. (1996) the proportion of Caucasians was higher in patients than in controls, and it is possible that this difference affected the results. The risk of PD in first-degree relatives of Caucasians was 2.4-fold higher than that in first-degree relatives of African-Americans and Hispanics (Marder et al. 1996).

Differences in sex and age distribution may also complicate the analyses in case-control studies. The likelihood of manifesting PD increases with age (de Rijk et al. 1997, Twelves et al. 2003) and has been found to be higher in males (Fall et al. 1996, Kuopio et al. 1999, Baldereschi et al. 2000). In the present study, the sex distribution was similar in patients and controls, but controls were younger than the patients. However, this did not cause errors in the results since the age of the parents and siblings of the controls was not different from that of the patients. In addition, the age-dependent risk of PD was controlled for by comparing the lifetime cumulative incidence of PD between the patients and the controls.

The validity of the family history was considered in our study. Since family history information concerning PD has been evaluated as being highly sensitive and specific, verification of cases reported to be healthy was not done (Marder et al. 1996). Verification of secondary cases, which was based on review of medical records, showed a low number of false positive cases in our study reflecting good specificity in family history data of PD. Diagnostic variability caused by multiple interviewers was avoided as all interviews and chart reviews were performed by the author. A telephone interview was used instead of a questionnaire to get more detailed information about relatives and a better participation rate. Although efforts were made to ensure collection of accurate information about the disease status of relatives the existence of ascertainment bias and recall bias in our data cannot be ruled out. These may cause underestimation of the frequency of PD in relatives of controls. Relatives of patients with PD may be more likely than average to seek medical attention for the disease. The presence of relatives with PD might be recalled more frequently by patients than controls. The family study design would have provided more accurate data, but it would have been expensive and it is vulnerable to selection bias because of the restriction to those relatives who can be interviewed directly.

Diagnostic accuracy is also a challenge in epidemiological studies of PD. Studies may be confounded if patients with atypical parkinsonism are included. In a recent Norwegian study, patients with PD were classified in three groups based on the certainty of a correct diagnosis of PD: clinically definite, probable and possible. In patients with a high degree of certainty for the diagnosis the familial aggregation of the disease seemed stronger than in patients with lower diagnostic accuracy (Kurz et al.

2003). These results suggest that the study may underestimate the genetic component of PD if strict diagnostic criteria are not used. The Finnish health care system provides an advantage regarding the diagnosis of PD since diagnosis in most cases is done by neurologists. Our subjects satisfied accepted diagnostic criteria (Daniel and Lees 1993) and this should ensure that the sample included patients with idiopathic PD and not atypical parkinsonism.

6.1.2. Segregation analysis

The purpose of segregation analysis is to elucidate of types of genetic effects that underlie familial aggregation of PD. Apart from our study, only two segregation analyses in connection with PD have been conducted (Zareparsi et al. 1998, Maher et al. 2002). Since both of these studies recruited patients from a specialty movement disorder clinic, our study represents the first segregation analysis in a population-based sample of patients with PD. Zareparsi et al. (1998) correctly emphasise that their findings may not be applicable to all PD populations and they need to be confirmed in other clinic- and population-based studies.

The segregation analyses by Zareparsi et al. (1998) and Maher et al. (2002) used regressive logistic models for disease (Bonney 1986). In the present study, segregation analysis was based on the so-called mixed model, which postulates that a disease may be explained by the genetic contributions of a single major locus, or be a polygenic nature, or both. The mixed model can discriminate between Mendelian inheritance and polygenic inheritance and also analyzes for reduced penetrance. Moreover, it is possible to test for an etiological heterogeneity in a given trait which was not done in the other studies (Zareparsi et al. 1998, Maher et al. 2002). This is important since the clinical presentation is variable and the role of genetic susceptibility may vary among subgroups defined by clinical features such as age at onset. We conducted the analyses in subgroups of EOPD and LOPD finding evidence of genetic heterogeneity.

As in familial aggregation studies, problems in the validity of the family history may complicate segregation analysis. The reduced ascertainment of PD gene carriers among the parents may make it impossible to show the existence of a major gene effect even though one truly exists. It is also important to keep in mind that CSA could potentially reject Mendelian inheritance when the sample is a mixture of two major

genes with different mode of inheritance (Jarvik 1998). In addition, CSA is unable to distinguish between the effect of a single locus and two or more independently acting loci with similar transmission patterns (Jarvik 1998). Given that both ADPD and ARPD are associated with several genes and loci, both of these effects are quite possible also in our sample.

6.1.3. Molecular genetic analyses in familial PD

Our molecular genetic study on familial PD failed to find mutations in the α-synuclein and the parkin gene. Non-random ascertainment of patients with familial PD and the small number of families does not permit us to exclude the involvement of these genes in familial PD in the whole Finnish population. However, mutations in the α-synuclein gene appear to be a rare cause of ADPD. Scott et al. (1999) found the Ala53Thr mutation in the α-synuclein gene in 1 of 186 families with multiple affected patients with PD, giving a frequency 0.5 percent for this mutation. In contrast, the frequency of parkin gene mutations has been found to be 50 percent in European families with EOPD (Lücking et al. 2000).

Finding a causative gene for PD would be easier if it were possible to investigate large pedigrees with multiple affected cases. As many studies have shown, linkage analysis in those cases is a powerful method (Polymeropoulos et al. 1996, Matsumine et al. 1997, Gasser et al. 1998, Farrer et al. 1999a, Valente et al. 2001, van Duijn et al. 2001, Funayama et al. 2002). The Icelandic genome screening study has demonstrated that the genome screening of multiple extended pedigrees is another successful strategy (Hicks et al. 2002, Gulcher et al. 2001). Given that our CSA suggested the presence of a major gene in Finnish PD families it would be interesting to use the same strategy in Finland. The genealogical data from the population registers of the Lutheran Church might allow the extension of Finnish pedigrees large enough for linkage analysis.

6.1.4. Analysis of mitochondrial DNA polymorphism

Several studies have attempted to examine the association between mtDNA polymorphism and PD. Polymorphism 4336A>G has suggested increasing the risk of AD and PD (Shoffner et al. 1993). Recently, an association was reported between 10398A>G in the MTND3 gene and reduced risk of PD (van der Walt et al. 2003).

These studies have highlighted the role of a single polymorphism, which may be an erroneous conclusion. We must bear in mind that mtDNA is a haploid, nonrecombining genome with a high mutation rate, and the polymorphisms in mtDNA occur in lineages and are accompanied by other polymorphisms in that lineage. All individuals belonging to haplogroup subcluster H4 carry 4336A>G polymorphism. The polymorphism 10398A>G in turn is contained within haplogroups I, J and K. There are examples from other complex diseases providing evidence that the increased risk of a phenotype could be due to a combination of polymorphisms rather than a single polymorphism. Certain combinations of otherwise harmless polymorphisms in mitochondrial lineages may increase susceptibility to disease (Wallace et al. 1999, Chinnery et al. 2000, Ruiz-Pesini et al. 2000) and increased sequence variation in itself may be a genetic risk factor for sensorineural hearing impairment (Lehtonen et al. 2003).

Statistical analyses of human mtDNA sequences have revealed a variety of non-neutral patterns (Moilanen and Majamaa 2003). The variation in the MTND genes was also found to be non-neutral, suggesting that many amino acid mutations are slightly deleterious. Mildly deleterious mutations would be compatible with life, but mitochondrial function declines with age in postmitotic tissues and lead to OXPHOS deficiency and ultimately to clinical symptoms (Wallace 1995). Given that the evidence for complex I dysfunction in PD is convincing, there are good reasons to expect that sequence variation in MTND genes contribute to the risk of PD. Sequence analyses of MTND genes in patients with PD have failed to find evidence to support this (Kösel et al. 2000, Simon et al. 2000, Vives-Bauza et al. 2002). However, they do not exclude the importance of sequence variation in MTND genes in PD, since sample sizes in these studies have been small. Our hypothesis was that the risk of PD is conveyed by the total number of nonsynonymous substitutions in the MTND genes in various mtDNA lineages rather than by single mutations. To test this hypothesis, we determined the nonsynonymous to synonymous substitution ratio (K_a/K_s) of MTND genes using sequence data from 183 Finns (Finnilä et al. 2001a). A statistical approach was used to analyze whether there are differences in the K_a/K_s ratio of the seven MTND genes between the European haplogroup clusters. The association between a high K_a/K_s ratio and susceptibility to PD or clinical features was then investigated in the case-control study. We included 210 patients with PD, 28 patients with PDD and 104 controls for

analyses, but unfortunately the sample sizes were too small to reach statistical power in risk ratio analyses. The division of PD patients into two groups (non-demented and demented) also reduced statistical power in the risk ratio analyses, but it was the right procedure since the PDD group differed significantly from the PD group in many analyses.

6.2. Genetics of Parkinson's disease in Finnish population

6.2.1. Familial aggregation

Although none of the population-based case-controls studies used the same methods as the present study in case ascertainment, collection of family history information and case verification, four of the studies are sufficiently similar to merit comparison of results (Semchuk et al 1993, Marder et al. 1996, Elbaz et al. 1999, Kuopio et al. 2001). The relative risk of PD was 2.9-fold and the cumulative incidence of disease by the age 90 years was 3.3-fold higher among the first-degree relatives of patients than those of controls. Given that the other studies have reported a relative risk of 2.1-3.2 for PD in first-degree relatives, a remarkable similarity in the results is found across studies. Familial aggregation of PD in Finland is similar to that in other populations. These results imply strong support for a genetic role in PD.

Familial aggregation does not necessarily imply a genetic etiology. Alternative explanations include behavioural risk factors shared by family members (diet, smoking) and common environmental exposures in family members living together (well water drinking, farming, pesticides). However, the results of our segregation analysis as well as two other segregation analyses strongly rejected the environmental model (Zareparsi et al., Maher et al. 2002). A recent Icelandic study based on genealogic information of the whole population demonstrated that the familial aggregation of PD extends beyond the nuclear family (Sveinbjornsdottir et al. 2000). Furthermore, in the same study, as in others, the prevalence of PD among spouses of patients with PD was not higher, suggesting that a shared environmental factor, late in life, is unlikely to explain the familial aggregation of PD (Payami et al. 1994, Sveinbjornsdottir et al. 2000, Maher et al. 2002). There is no convincing evidence that environmental factors early in life contribute to risk of PD (Martyn et al. 1995). These findings strongly indicate that the familial aggregation in PD is caused largely or exclusively by genetic factors.

6.2.2. Mode of inheritance

Both autosomal dominant and autosomal recessive transmission in PD are known to occur. Maternal inheritance and genetic anticipation due to unstable trinucleotide repeat have also been proposed. In the present study, the CSA of total series, sporadic model was strongly rejected and none of the three major locus models explained the observed segregation pattern better than the polygenic model. The analysis show that a Mendelian locus is likely to exert a large effect in the subset of families with EOPD. This is consistent with the finding that the known causative genes for PD are all associated with early onset of the disease (Polymeropoulos et al. 1997, Kitada et al. 1998, Leroy et al. 1998, Lücking et al. 1998, Abbas et al. 1999, Papapetropoulos et al. 2001, Bonifati et al. 2003). Our results are also in agreement with those of a recent twin study suggesting that a major genetic susceptibility may be involved in EOPD (Tanner et al. 1999). However, our results do not agree with the hypothesis that no genetic susceptibility is involved in LOPD. Based on the results of our CSA our explanation for the existence of LOPD is polygenic inheritance.

The Finnish population is genetically homogenous and the entire population has evolved from a small founder element, which has resulted in enrichment of otherwise rare disease alleles, a phenomenon known as the Finnish heritage (Norio et al. 1973, Norio 2003). It has been suggested that majority of cases of PD in Finland might even result from a single founder mutation (Farrer et al. 1999b). Our results do not support this suggestion, since no major gene effect was found in the analysis of the total data set. The results of our CSA points the existence of a major gene in familial PD. However, it was not possible to distinguish between a recessive model with a high penetrance and a dominant model with lower penetrance. Incomplete penetrance is possibly related to all loci linked to ADPD, since asymptomatic carriers older than the expected age at onset of PD have been observed in these families (Leroy et al. 1998, Gasser et al. 1998, Farrer et al. 1999a, Papadimitriou et al 1999, Funayama et al. 2002). It has been suggested that this low penetrance may be caused by environmental or other genetic factors modifying the expression of the disease (Funayama et al. 2002). On the other hand, incomplete penetrance is a typical feature in autosomal dominant disorders.

The success of gene discovery studies depends on a fundamental knowledge of the mode of inheritance. Parameters estimated for the recessive and dominant models may

be useful in parametric linkage analysis (Jarvik 1998). In addition, the results of our CSA may prove useful in identifying subgroups of patients with PD who are suitable for further gene discovery studies. Our results suggest that the discovery of mutations causing PD in the Finnish population are most probable if patients with EOPD are selected for molecular genetic studies.

6.2.3. Familial PD

The present study was the first Finnish molecular genetic study of familial PD. We were unable to detect mutations in the α-synuclein or parkin genes. This finding was inconsistent with those of other studies where various mutations in the parkin gene have been affirmed to be a major cause of autosomal recessive parkinsonism amongst European families (Abbas et al. 1999, Lücking et al. 2000). At the time when the molecular analyses were conducted in our sample, the oldest parkin-positive patient having an age at onset of 58 years reported by Abbas et al. (1999). The phenotype of EOPD due to parkin pathology in that sample was indistinguishable from that of typical late-onset PD (LOPD). However, it is notable that PD patients with parkin mutations in that sample showed a phenotype of EOPD with mean age at onset of 38 ± 12 years. In the study of Lücking et al. (2000) the mean age at onset in the patients with parkin mutations was younger than that in those without mutations (32 ± 11 vs. 42 ± 11 years). In the present study the sample of patients with familial PD represents typical LOPD with the mean age at onset of 57 ± 11 years even in the families classified as EOPD families. Only a few studies analyzing the parkin gene have included patients with LOPD (Oliveri et al. 2001, Kann et al. 2002, Foround et al. 2003). Although parkin mutations have been recently identified in patients with LOPD (Foroud et al. 2003), the LOPD cases with parkin mutation are still unusual, and also in this LOPD sample those without a parkin mutation have older age at onset of PD compared with parkin mutation-positive cases (60.7 vs. 50.3, Foroud et al. 2003). Thus, it is probable that parkin pathology influences the age at onset of parkinsonism, and it is conceivable that familial and sporadic LOPD could mainly be due to causes other than parkin mutations (Oliveri et al. 2001). In agreement with this are the results of a complete genomic screen in familial PD providing evidence that the parkin gene is important in the development of EOPD and multiple genetic loci influence the susceptibility of LOPD (Scott et al.

2001). On the basis of these findings, it seems probable that parkin mutations in our patients group which consisted mostly of LOPD patients would be rare indeed. For these reasons we do not consider it worthwhile examining the parkin gene further in the sample of familial PD patients. Instead, it might be more likely that an associated locus be found elsewhere in the genome.

Several studies have consistently observed that cases of familial and sporadic PD display similar clinical features (Plante-Bordeneuve et al. 1995, Carr et al. 2003). The same was observed in the present study. This may suggest similar pathogenesis for both familial and sporadic PD.

6.2.4. Mitochondrial DNA polymorphism and susceptibility to PD

Several reports have suggested that the oxidative stress and OXPHOS dysfunction may play a role in the pathogenesis of PD. Dysfunction of complex I in PD is well established. Except for MPTP and rotenone, causes of complex I dysfunction are poorly understood (Betarbet et al. 2000, Nicklas and Heikkilä 1985). Other potential causes are mutations or polymorphisms of MTND genes or genes in nuclear DNA encoding subunits of complex I. One finding suggesting nuclear DNA involvement is that the polymorphism in the NDUFV2 gene is associated with increased susceptibility to PD (Hattori et al. 1998). However, the seven MTND genes have homologues among the 14 genes that encode bacterial complex I, indicating that the mtDNA encoded subunits are indispensable for the function of the respiratory chain (Smeitink et al. 1999). Some studies have provided evidence that mutations in mtDNA could contribute to complex I defect in PD (Swerdlow et al. 1996, Swerdlow et al. 1998, Gu et al. 1998), but other studies have failed to identify specific sequence changes in MTND genes associated with PD (Ibeke et al. 1995, Bandmann et al. 1997, Kösel et al. 1998, Kösel et al. 2000, Simon et al. 2000, Richter et al. 2002). The entire mitochondrial genome has recently been sequenced from the SN of 8 patients with PD and 9 controls (Vives-Bauza et al. 2002). Several previously reported polymorphisms differed between PD patients and controls, but the total number of sequence variants did not differ between PD patients and controls. The number of subjects in this study was small, analyses were not done in a case-control design, and the groups were not matched with respect to haplogroup. Bearing in mind these limitations, the results must be interpreted with caution.

Recently, two studies have suggested that mtDNA polymorphisms in MTND genes may modify the risk of PD. The 10398G>A polymorphism in MTND3 defining European haplogroups I, J and K has been suggested to be associated with a reduced risk of PD (van der Walt et al. 2003). Another study has suggested that 4216T>C in MTND1 defining haplogroup cluster JT is more frequent among patients with PD than among healthy aged controls (Ross et al. 2003). Our findings are in agreement with those of the latter study, as we found that supercluster JTIWX was more frequent among PD patients and even higher among patients with PDD. The discrepancies between the results of the three studies may be related to problems in the selection of cases and controls. The Irish (Ross et al. 2003) and the Finns are homogeneous populations, whereas a similar homogeneity is difficult to attain in selecting cases from the US population. MtDNA sequence diversity has been shown to vary greatly between populations. The modal value of pairwise nucleotide differences in non-African populations is 28-32 (Ingman et al. 2000), whereas the mean of pairwise differences in Finns is as low as 21 (Finnilä et al. 2001). Any attempt to compare mtDNA variation between patients and controls should thus be carried out in a population with a fairly homogeneous ethnic background.

Our analysis revealed that the haplogroup clusters differed in the K_a/K_s ratio, clusters HV and KU having a lower ratio than clusters JT and IWX. We found that the frequencies of mtDNA haplogroup clusters in PDD patients differed from those in PD patients and controls. Supercluster JTIWX with a high K_a/K_s ratio was more frequent among PD patients and even more frequent among patients with PDD. Furthermore, patients belonging to haplogroup I, J or K had a slightly increased risk of PDD and a significantly lower age at onset of PDD compared with those belonging to the remaining European haplogroups. Complex I dysfunction may thus increase the risk of PD and, moreover, increase the risk of disease progression to dementia. Diffuse or transitional Lewy body disease is the most important pathologic correlate of dementia developing in PD (Hurtig et al. 2000, Mattila et al. 2000, Apaydin et al. 2002). Clinical symptoms of PDD overlap with dementia with Lewy bodies (McKeith et al. 1996, Aarsland et al. 2003). The diagnosis of DLB could not be ruled out in the patients with PDD in this study, although none of our study subjects fulfilled the clinical criteria for DLB.

The present study is the first to demonstrate that the haplogroup clusters differ in the K_a/K_s ratio of MTND genes, which may contribute to neurodegeneration. Although the odds ratio analysis does not reach statistical significance, there is an obvious tendency for increased risk of PD and disease progression to dementia in supercluster JTIWX with high a K_a/K_s ratio. Therefore, further research is warranted to confirm the contribution of nonsynonymous mutations in MTND genes to the complex I dysfunction in PD.

7. CONCLUSIONS

1) The increased relative risk of PD and the cumulative incidence among first-degree relatives of patients with PD demonstrate that familial aggregation of PD exists in the Finnish population as in other populations.

2) a) The results of the segregation analysis indicate that the etiology of idiopathic PD is heterogenous even in the Finnish population, which has evolved from a small group of founders.

b) The analysis indicate that the familial aggregation of PD is caused by genetic factors.

c) Significant heterogeneity was found between EOPD and LOPD, suggesting that the contribution of major genes is higher in EOPD than in LOPD.

d) The analysis of familial PD supports the hypothesis that a major locus is present in this subset, but it was not possible to distinguish between a recessive model with a high penetrance and a dominant model with a relatively low penetrance.

3) The sequence analysis of the α-synuclein gene and mutation analysis of the parkin gene do not support the association of mutations in these genes with familial PD in our sample.

4) a) The analysis of the nonsynonymous to synonymous substitution rate ratio (K_a/K_s) of MTND genes in Finns revealed that the haplogroup clusters differed in the K_a/K_s ratio, clusters HV and KU having a lower ratio than clusters JT and IWX.

b) Supercluster JTIWX with a high K_a/K_s ratio was more frequent among PD patients compared with controls and even more frequent among patients with PDD, suggesting that a relative excess of nonsynonymous mutations in MTND genes in supercluster JTWIX is associated with increased risk of PD and disease progression to dementia.

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