Introduction

Parkinson disease is a clinically defined syndrome, characterized by variable combinations of akinesia, rigidity, tremor and postural instability. These symptoms are caused by a degeneration of dopaminergic neurons of the substantia nigra, leading to a deficiency of dopamine in their striatal projection areas. Characteristic eosinophilic inclusions, the Lewy bodies, are found in surviving dopaminergic neurons but also, though less abundantly, in other parts of the brain, and have been considered to be essential for the pathologic diagnosis of PD.

Genetic research of the past years, in particular the mapping and cloning of a number of genes which cause, when mutated, monogenically inherited forms of the disorder has shown that PD is actually not a disease entity, but rather a heterogeneous group of diseases associated with a spectrum of clinical and pathological changes.

A minority of patients with the typical clinical picture of PD have a positive family history compatible with a Mendelian (autosomal dominant or autosomal recessive) inheritance. As a rule, age at onset in many (but not all) of these patients is younger than that of patients with sporadic disease, but no other specific clinical signs or symptoms distinguish familial from sporadic cases. Pathologically, all forms have in common a predominant degeneration of dopaminergic neurons of the substantia nigra, although in some forms the degenerative process is more widespread. In some forms Lewy bodies are found in surviving dopaminergic neurons of the substantia nigra, leading to a deficiency of dopamine in their striatal projection areas. Characteristic eosinophilic inclusions, the Lewy bodies, are found in surviving dopaminergic neurons but also, though less abundantly, in other parts of the brain, and have been considered to be essential for the pathologic diagnosis of PD.

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Remarkably, pathology can even vary within single families. These observations indicate that different, and probably interrelated pathogenic pathways are likely to lead to the process of nigral cell death.

Results/Project Status

Role of α-synuclein in sporadic PD

The first "PD gene" to be recognized was the gene for α-synuclein (1). Only three different point mutations have been recognized, all causing an autosomal-dominant form of PD with high penetrance. A direct causal link between α-synuclein and PD is supported by the recent discovery that not only point-mutations, but also multiplications of the wildtype α-synuclein gene (duplications and triplications) cause autosomal-dominant parkinsonism with or without dementia with α-synuclein inclusions (2) in some families. This finding is of mechanistic importance because it indicates that a mere increase in α-synuclein levels, which can also be measured in the blood in cases with triplications can be toxic to neurons. On a population level, dosage changes of SNCA are again a rare cause of dominant parkinsonism and are very rare in the sporadic disease. This was shown within the NGFN in a study of 54 patients with diffuse Lewy body disease (DLB) and 104 patients with early-onset PD (3).

The recognition of the relevance of α-synuclein expression levels have revitalized studies looking at polymorphisms in SNCA, which might influence expression levels, in the sporadic disease. Earlier results had been controversial. Some, but not all studies found a complex polymorphic dinucleotide repeat polymorphism (NACP-Rep1) located about 4 kb upstream of the transcriptional start site of SNCA to be associated with sporadic PD.

The testable hypothesis is that strong overexpression of α-synuclein, as caused by duplications or triplications of the gene, lead to autosomal-dominantly inherited Lewy-body parkinsonism (with or without dementia, depending on the degree of overexpression) with early onset, whereas slight but chronic overexpression which may be caused by variations in the regulatory sequences of the gene, may increase the risk to develop the disease to a certain degree, but not strongly enough to be detectable as an inherited Mendelian trait. Within the framework of the NGFN, we addressed this question by a more general approach. We first defined the haplotype structure of the entire SNCA-gene by analyzing more than 50 SNP-pairs across the gene. The gene is contained within two major haplotype blocks (Fig. 1). In a group of 340 sporadic German patients with PD and 680 controls derived from the KORA-population, SNP-markers within the 3’-block around exons 5 and 6 showed strong association with Parkinson’s disease (p<0.00009), conferring a relative risk of about 1.4 in heterozygous carriers of the risk haplotype and about 2 in homozygotes. Taking into account the population frequencies of the respective “risk” or “protective” haplotypes, this corresponds to a population attributable risk of approximately 29 and 42%, respectively. The association was confirmed in a second sample of similar size. Effects of the associated variants might be mediated by regulatory elements in this highly conserved region or by a frequency shift in a previously described splice variant lacking exon 5. A direct association with promoter polymorphisms could not be replicated in our sample set.

![Fig 1: Linkage disequilibrium (LD)-structure of the alpha-synuclein: red color denotes areas of high LD, green and blue stands for low LD. The SNCA-gene (white arrow) is contained within 2 LD-blocks (read squares).](image)

Subsequent in vitro studies have indicated that at least one of the SNP-variants identified affect expression of a reporter gene in HEK-cells (Fuchs et al., unpublished). Other potential mechanisms, including alterations of RNA-stability or an effect on translation are presently under investigation.

Identification of LRRK2, the most common gene for dominant PD

A second locus for a dominant form of PD has been mapped in a large Japanese family to the pericentromeric region of chromosome 12. Affecteds in this family showed typical L-dopa responsive parkinsonism with onset in their fifties. Pathologically, nigral degeneration was found, but no Lewy bodies or other distinctive inclusions.

Project: Identification of Genetic Risk Factors of Parkinson's Disease

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Disease-oriented Genome Networks

Based on this finding, in a collaboration within NGFN2 and with a group at the Mayo Clinic Jacksonville, USA, we refined the mapping of this locus in two families of European descent to a region of approximately 20 cM (4). Following a positional cloning approach we successfully identified the disease gene in these and several other families with dominantly inherited PD: The disease is caused by point mutations in the gene for leucine-rich repeat kinase 2 (LRRK2) (5). The encoded protein has also been called “dardarin” (6). The gene spans a genomic region of 144 Kb, with 51 exons encoding 2527 amino acids (Figure 2). The gene is expressed in all brain regions and also in all peripheral tissues examined so far, although at low levels. LRRK2-associated PD is remarkable for several reasons. First, mutations in the LRRK2 gene appear to be the most common cause of autosomal-dominantly inherited parkinsonism discovered so far. Four different mutations were detected in five of 34 dominant families studied by Zimprich et al. (5) (in two of the families, the same mutation, R1441C, arose independently, based on the analysis of polymorphisms closely surrounding the gene). The same codon was affected in the group of Basque families studied by Paisan-Ruiz et al. (6), but this mutation resulted in a different amino-acid exchange. These findings indicate that mutations in LRRK2 are responsible for approximately 10 to 20% of dominantly inherited PD. Subsequently, a particularly common mutation, Gly2019Ser, was detected on a founder haplotype across several European populations and in up to 20% of dominantly inherited PD. Subsequently, a particularly common mutation, Gly2019Ser, was detected on a founder haplotype across several European populations and up to 5 to 6% of several large cohorts of families with dominant parkinsonism (7, 8), and even in 1 to 2% of patients with sporadic disease (9). Remarkably (and in contrast to all previously described forms of monogenic PD), both familial and sporadic cases were indistinguishable from typical sporadic PD as far as their age of onset was concerned. Given the total prevalence of typical late-onset PD, LRRK2-related PD may well be as common as other well-known inherited neurodegenerative disorders, such as Huntington’s disease or the spinocerebellar ataxias.

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Second, mutations in the LRRK2 gene are particularly interesting because they may affect several different amino acids. These findings indicate that mutations in LRRK2 are responsible for approximately 10 to 20% of dominantly inherited PD. Subsequently, a particularly common mutation, Gly2019Ser, was detected on a founder haplotype across several European populations and in up to 5 to 6% of several large cohorts of families with dominant parkinsonism (7, 8), and even in 1 to 2% of patients with sporadic disease (9). Remarkably (and in contrast to all previously described forms of monogenic PD), both familial and sporadic cases were indistinguishable from typical sporadic PD as far as their age of onset was concerned. Given the total prevalence of typical late-onset PD, LRRK2-related PD may well be as common as other well-known inherited neurodegenerative disorders, such as Huntington’s disease or the spinocerebellar ataxias.

Finally, although the clinical picture appears to resemble typical PD in most cases, the associated pathology is remarkably variable. Pathologic changes include abnormalities consistent with Lewy body Parkinson’s disease, diffuse Lewy body disease, nigral degeneration without distinctive histopathology and progressive supranuclear palsy-like tau aggregation. LRRK2 mutations may therefore be an upstream event in the cascade leading to neurodegeneration with different pathologies. The function of the encoded protein is still unknown. By sequence homology, LRRK2 can be assigned to the group of recently identified ROCO-proteins (10) and contains a protein kinase domain of the MAPKKK class, suggesting a role in intracellular signalling pathways. Mutations appear to be clustered in functionally important regions, which are highly conserved through the species.

It is therefore interesting to speculate that the molecular pathway of LRRK2-signalling may be involved in a general neuroprotective function, which is pertinent to more than one form of neurodegeneration. This pathway may well lend itself to pharmacological manipulation, which would provide a novel target for neuroprotective drugs.

Outlook

Although genes that are linked to monogenic forms of Parkinson’s disease and other closely related neurodegenerative diseases are, at first glance, not related to a common cause, recent genetic, pathologic and molecular studies have strengthened the evidence that there is probably more “cross-talk” between the different pathways on several levels than previously appreciated. These findings support the existence of common pathogenic mechanisms, including protein aggregation, mitochondrial dysfunction or oxidative stress, which had been suspected as major culprits of neurodegeneration for many years. It strengthens the notion that the study of genes identified in rare monogenic forms of neurodegenerative disorders is also fruitful for the identification of the causes of the common sporadic forms. An in depth study of disease mechanisms associated with those genes, in combination with a careful selection of other candidate genes as well as with total genome screening approaches will provide the basis for further exciting developments in this field, which will hopefully provide the basis for novel treatment strategies.