Update on the Genetics of Parkinson’s Disease

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Abstract: Over the last few years, several genes for monogenic forms of Parkinson’s disease (PD) have been mapped and/or cloned. Mutations have been identified in the gene for α-synuclein in rare families with dominant PD, indicating that aggregation of this protein in Lewy bodies is probably a crucial step in the molecular pathogenesis of the disorder. A much more common cause for dominant PD, mutations in the gene for leucine-rich repeat kinase 2 (LRRK2), has recently been identified. Mutations in the parkin gene, in DJ-1 and PINK1 all cause autosomal recessive parkinsonism of early onset. These genes have been implicated in the proteasomal protein degradation pathway, in the oxidative stress response and mitochondrial function. Mutations in recessive genes probably are pathogenic through loss-of-function mechanisms, suggesting that their wildtype products protect dopaminergic cells against a variety of insults. Evidence is emerging that at least some of these genes may play a direct role in the etiology of the common sporadic form of PD. Further, it is likely that the cellular pathways identified in rare monogenic variants of the disease also shed light on the molecular pathogenesis in typical sporadic PD. © 2007 Movement Disorder Society

Key words: Parkinson disease; genetics; LRRK2; synuclein; parkin

Parkinson disease (PD) is a clinicopathologic construct defined by a clinical syndrome with variable combinations of akinesia, rigidity, tremor and postural instability, and a characteristic pattern of neurodegeneration, predominantly, but not exclusively, 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. Although the different mutations and loci identified so far appear to be directly responsible in only a relatively small number of families each, there is accumulating evidence that the molecular pathways identified may be common to more than one genetic form of Parkinsonism and may, in one way or another, also play a role in the common sporadic disease. This will, eventually, allow to the development of novel protective and therapeutic strategies.

MONOGENIC FORMS OF PARKINSON’S DISEASE

Approximately 10–15% 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.

Genes Causing Autosomal Dominant Forms of Parkinsonism

To date, at least two genes, α-synuclein (α-SYN), and recently, leucine-rich repeat kinase 2 (LRRK2) are known to cause autosomal-dominant PD. The status of a
third gene, ubiquitin C-terminal hydrolase L1 (UCHL1 or PARK5) is still somewhat controversial. These discoveries have proven to be extremely fruitful.

**PARK1/4: α-SYN**

The first “PD gene” was mapped to the long arm of chromosome 4 in a large family with dominant inheritance and Lewy body pathology, and identified as the gene for α-SYN. Over the years, only three different point mutations have been recognized, each representing a single mutational event, all in large, multigenerational families. α-SYN point mutations have not been found in sporadic PD.

Although α-SYN mutations are rare, their identification was extremely important, as it lead to the discovery that the encoded protein is the major fibrillar components of the Lewy body, the proteinaceous inclusion which has, since Friedrich Lewy’s original description in 1917, been considered to be the pathologic hallmark of PD in both familial and sporadic cases.

The currently favored hypothesis states that the amino acid changes in the α-SYN protein associated with PD may lead to an increased tendency to form aggregates, although the precise relationship between aggregation and cellular dysfunction and cell death is unknown.

A direct link between α-SYN and PD is supported by the recent discovery that not only point mutations, but also multiplications of the wildtype sequence of the α-SYN gene (duplications and triplications) cause autosomal-dominant parkinsonism with or without dementia with α-SYN inclusions, indicating that a mere increase in α-SYN levels, which can also be measured in the blood in cases with triplications can be toxic to neurons.

The clinical picture in the affected subjects from pedigrees with α-SYN mutations or multiplications ranges from typical idiopathic PD to dementia with Lewy-bodies, although age at onset is lower (mean of about 35–45 years with a wide range) and progression appears to be more rapid than in sporadic cases.

The role of genetic variations in the α-SYN gene in the etiology of sporadic PD has recently been investigated in more detail. Point mutations and gene dosage mutations have not been found in sporadic patients. Several recent studies have addressed the question whether polymorphisms in the α-SYN gene may be associated with the sporadic disease. Earlier results had been controversial. Some, but not all studies found a complex polymorphic dinucleotide repeat polymorphism (NACP-Rep1) located in the promoter region to be associated with sporadic PD. In a more general approach, Müller and coworkers first defined the haplotype structure of the entire SNCA-gene by analyzing more than 50 SNPs across the gene. They found a strong association of a haplotype block comprising exons 5 and 6 and the 5’-UTR of the SNCA gene with PD, conferring a relative risk of about 1.4 in heterozygous carriers of the risk haplotype and about 2 in homozygotes. It is of course likely that more than one mechanism regulates α-SYN expression. Specific binding of a nuclear protein, poly(ADP ribose) polymerase-1 (PARP-1) to the NACP-Rep1-sequence was recently discovered and may be responsible, at least in part, for the association of specific alleles of this repeat sequence with PD. If confirmed, pharmacologic manipulation of α-SYN expression may be a possible therapeutic or preventive strategy in susceptible individuals. α-SYN is a relatively small protein that is abundantly expressed in many parts of the brain and localized mostly to presynaptic nerve terminals. Many aspects of the normal function of α-SYN are still unknown. The protein has been shown to bind to brain vesicles and other cellular components and may be functionally involved in brain plasticity. However, knockout mice for α-SYN show only very subtle alterations in dopamine release under certain experimental conditions, but no other phenotype.

There is still no good explanation for the striking selectivity of neuronal damage, which is prominent in dopaminergic cells whereas α-SYN is abundantly expressed in many areas of the brain. The identification of proteins that specifically bind α-SYN, like synphilin, may shed new light on this important question. In addition, several animal models have recently been described. Transgenic mice overexpressing α-SYN, both the normal and the mutated human sequence, under different promoters show accumulation of the protein but do not mimic the full degenerative process in the substantia nigra. A transgenic Drosophila model may be more true to the human disease. Interestingly in this model, pathology appears to be mediated by mitochondrial dysfunction, at least in muscle tissue, and can be partially rescued by coexpression of molecular chaperones.

**PARK8: Leucine Rich Repeat Kinase 2 (lrrk2)**

Another locus for a dominant form of PD has been mapped in a large Japanese family to the pericentromeric region of chromosome 12 and named PARK8. Affected in this family showed typical levodopa (l-dopa) responsive parkinsonism with onset in their fifties. Pathologically, nigral degeneration was found, but no Lewy bodies or other distinctive inclusions.

Recently, the PARK8, gene has been identified: the disease is caused by point mutations in the gene for LRRK2. The encoded protein has also been called “dardarin.” The gene spans a genomic region of 144
The gene spans a genomic distance of 144 kb and contains 51 exons. LRR: leucine rich repeat; Roc: Ras of complex proteins; COR: C-terminal of Roc; MAPKKK: mitogen activated kinase kinase kinase; WD: Beta-Propeller.

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. In the initial study, four different mutations were detected in 5 of 34 dominant families studied by Zimprich et al. (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., but this mutation resulted in a different amino-acid exchange. The proportion of about 10–20% of dominant families carrying mutations in **LRRK2** has been confirmed in subsequent studies, although comprehensive systematic mutation screens of the entire large gene have not yet been published.

One particularly common mutation, Gly2019Ser, was detected on a founder haplotype across several European populations and in up to 5–6% of several large cohorts of families with dominant parkinsonism, and even in 1–2% of patients with sporadic late-onset disease. Even more remarkable, in some genetically isolated populations such as the Ashkenazi Jewish and the North African Berber Arabs, mutation frequencies in both familial and sporadic cases of up to 20 to 40% have been found.

Second, clinical signs and symptoms resemble typical sporadic PD in most families. This is true also for age of onset, which is on average in the late fifties and sixties in the families described so far. Therefore, of the PD-genes identified so far, **LRRK2** mutations are by far the most common genetic cause of inherited PD and are likely to play a role also in the setting of typical sporadic late-onset disease.

Third, although the clinical picture appears to resemble typical PD, the associated pathology is remarkably variable. Pathologic changes are consistent with typical Lewy body PD in most cases reported so far, but also include 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.

By sequence homology, **LRRK2** can be assigned to the group of recently identified ROCO-proteins and contains a protein kinase domaine of the MAPKKK class, suggesting a role in intracellular signaling pathways, but its precise function remains to be determined. Mutations appear to be clustered in functionally important regions, which are highly conserved through the species.

Current experimental evidence suggests that pathogenic mutations may be associated with an increased kinase activity (Gloeckner, 2006), providing the possibility that kinase inhibition may be a promising therapeutic option.

**Autosomal Recessive Forms of Parkinsonism**

One of the surprising developments of recent years was the recognition of the relatively high proportion of patients with early-onset parkinsonism caused by recessive mutations in several genes (Table 1). So far, three have been identified: **parkin (PARK2)**, **PINK1 (PARK6)**, and **DJ-1 (PARK7)**. Again, the study of the function of these genes has provided valuable insight into the molecular mechanisms of dopaminergic degeneration.

**PARK2: Parkin**

Juvenile cases of parkinsonism in siblings were first recognized in Japan. The first genetic locus for autosomal-recessive juvenile parkinsonism (AR-JP), as this form of PD was called, was mapped to chromosome 6.
Mutations were then identified in a large gene in that region that was called parkin. Clinically, these patients suffer from L-dopa-responsive parkinsonism and often develop early and severe L-dopa-induced motor fluctuations and dyskinesias. Some show diurnal fluctuations, with symptoms becoming worse later in the day. Dystonia at onset of the disease is common. Parkin mutations turned out to be a common cause of parkinsonism with early onset, particularly in individuals with evidence of recessive inheritance. Nearly 50% of families from a population of sibling pairs with PD had parkin mutations. Also, parkin mutations are responsible for the majority of sporadic cases with very early onset (before age 20), and are still common (25%) when onset is between 20 and 35. Prevalence is almost certainly well below 5% in those with onset later than 45. Several studies have described the clinical spectrum of parkin-associated parkinsonism. Mean age at onset in a European population was 32 years; progression of the disease was usually relatively slow, but L-dopa-associated fluctuations and dyskinesias occurred frequently. Dystonia (usually in a lower extremity) at disease onset was found in about 40% of patients, and brisk reflexes of the lower limbs were present in 44%. Psychiatric abnormalities have been recognized in PD patients with parkin mutations but there are no systematic studies to determine whether this is a characteristic feature associated with parkin-mutations. Phenotype–genotype studies implicate that the type of mutation may influence the clinical phenotype to a certain degree: patients with at least one missense mutation showed a faster progression of the disease with a higher UPDRS (United Parkinson’s Disease Rating Scale) motor score than carriers of truncating mutations. Missense mutations in functional domains of the parkin gene resulted in earlier onset.

It is still controversial whether heterozygous mutations in the parkin gene can cause parkinsonism or can confer an increased susceptibility for typical late-onset PD. There is evidence from imaging studies that heterozygous carriers of parkin mutations have reduced uptake of fluorodopa in the basal ganglia. Furthermore, families with heterozygous mutation carriers manifesting symptoms of PD have been described. On the other hand, the frequency of heterozygous mutations in the parkin gene was found to be similar in elderly healthy individuals, as compared to a cohort with late-onset typical PD, and in a large family reported recently, 12 heterozygous carriers of a particular parkin mutation (ex3delta40) were asymptomatic. Also, in a group of families with PD showing anticipation (late-onset PD in the parent generation and early-onset PD in the offspring) genotyping results did not support the explanation that the presence of single or compound heterozygous parkin-mutations contribute to this phenomenon. Therefore, at present the data are still insufficient to confidently judge the role of single heterozygous parkin mutations in the development of PD.

Knowledge on the neuropathology of molecularly confirmed cases of AR-JP is still based on only a few cases. Severe and rather selective degeneration of neu-

<table>
<thead>
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<th>Locus/gene</th>
<th>Inheritance</th>
<th>Onset</th>
<th>Pathology</th>
<th>Map position</th>
<th>Gene</th>
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<td>40s</td>
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<td>α-synuclein</td>
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<td>α-synuclein triplikations and duplications</td>
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Table I.

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rons in the substantia nigra and the locus coeruleus, usually with absence of Lewy bodies, has been described.\textsuperscript{53} However, a later publication found typical LBs in a single patient.\textsuperscript{49} In another case with parkin mutations, tau was found in the inclusions,\textsuperscript{54} and most recently, another case was reported showing α-SYN positive inclusions that resemble LBs in some respects, but differ in some of their staining properties.\textsuperscript{55} It is unclear whether these differences may be attributed to differential effects of parkin mutations on its E3-ubiquitin ligase function.

As mutations in parkin cause parkinsonism, in all likelihood by a loss-of-function mechanism, the study of the normal function of parkin should provide insight into the molecular pathogenesis of the disorder. Several groups have now shown that parkin, a protein found in the cytosol but also associated with membranes, functions in the cellular ubiquitination/protein degradation pathway as a ubiquitin ligase.\textsuperscript{56} It has been hypothesized that the loss of parkin function may lead to the accumulation of a nonubiquitinated substrate that is deleterious to the dopaminergic cell but, due to its nonubiquitinated nature, does not accumulate in typical Lewy bodies. Several proteins have been shown to interact with parkin. However, the putative toxic protein, which has been hypothesized to accumulate due to the lack of parkin in patients (or in knock-out animals, for that matter) has not yet been identified.

However, novel functions of parkin are being identified, and it is possible that they may be of equal or even greater relevance to the pathogenesis of PD. For example, it has been shown that parkin does not only mediate the well-studied ubiquitinylation via lysin48 (K48), which directs ubiquitylated proteins for proteasomal degradation, but also via lysin63 (K63), which may play a role intracellular signaling processes and also in Lewy body formation.\textsuperscript{57} Additional clues to possible other relevant functions of parkin have been derived from the proteomic analysis of parkin\textsuperscript{−}\textsuperscript{−}mice. A recent study revealed a decreased abundance of a number of proteins involved in mitochondrial function or oxidative stress, accompanied by a reduction in respiratory capacity of striatal mitochondria, a decreased serum antioxidant capacity and increased protein and lipid peroxidation.\textsuperscript{58} This corresponds well to recent analyses of Drosophila parkin\textsuperscript{−}\textsuperscript{−}models. Greene et al. extended an earlier study, that had detected mitochondrial pathology, apoptotic muscle degeneration, and locomotor defects in Drosophila parkin\textsuperscript{−}\textsuperscript{−}mutants\textsuperscript{52} and found that these changes are associated with a profound increase of expression of genes involved in the defense against oxidative stress\textsuperscript{59} and that loss-of-function mutations in genes for oxidative stress components enhance the parkin mutant phenotypes. These novel findings indicate that proteasomal dysfunction, although supported by several lines of evidence, may not be the sole mechanism contributing to neurodegeneration in parkin-related disease.

Whatever the mechanism, increasing evidence suggests an important role of parkin for dopamine neuron survival. Overexpression of wildtype rat parkin could protect against the toxicity of mutated human A30P α-SYN in a rat lentiviral model of PD. The parkin-mediated neuroprotection was associated with an increase in hyperphosphorylated α-SYN inclusions, suggesting a key role for parkin in the genesis of Lewy bodies.\textsuperscript{60}

PARK6: PINK1

Recently, mutations in the PINK1-gene (PARK6) have been identified as another cause for autosomal-recessive early-onset parkinsonism.\textsuperscript{6} This gene is particularly interesting within the context of the findings linking PD to mitochondrial dysfunction and oxidative stress, as PINK1 encodes a mitochondrially located protein. Mutations in the PINK1-gene are much less common than parkin mutations, and probably account for only 1–2% of early-onset cases.\textsuperscript{61–64} Again the question of the role of single heterozygous mutations is unsettled. In 5 of 100 patients studied by Valente et al.\textsuperscript{61} only a single mutation was identified. Age at onset in the heterozygotes was in the fourth to fifth decade (range, 37–47 years). Two of 200 healthy control individuals also carried one heterozygous missense mutation. Together with previous positron emission tomography studies demonstrating nigrostriatal abnormalities in clinically asymptomatic PARK6 carriers, this observation argues that haploinsufficiency of PINK1 may represent a susceptibility factor toward parkinsonism, but the question is certainly not settled.

PARK7: DJ-1

Mutations in the DJ-1 gene (PARK7) are another rare cause of autosomal-recessive parkinsonism.\textsuperscript{7,65,66} The clinical picture with early-onset and slow progression is similar to the other recessive Parkinson syndromes. Following the initial discovery of two mutations in an Italian and a Dutch family,\textsuperscript{7} only a few additional bona fide pathogenic mutations (one homozygous\textsuperscript{67} and one compound heterozygous\textsuperscript{68}) have been identified.

The normal function of DJ-1 and its role in dopamine cell degeneration is unknown, but there is evidence that links DJ-1 to oxidative stress response and mitochondrial function. Canet-Aviles et al. have shown that, in the presence of oxidative stress, wildtype DJ-1 translocates.
to the outer mitochondrial membrane and is associated with neuroprotection. Interestingly, DJ-1 is expressed mostly in astrocytes in normal and PD brain, stressing the importance of glial-neuronal interaction in PD.

Again, the pathogenic role of several single heterozygous sequence variants detected in cohorts of early-onset PD is unclear.

OTHER GENES AND LOCI

Several other loci have been mapped in families with PD, but either the genes have not yet been identified, or their role is still somewhat controversial.

A dominant locus has been described on chromosome 2p13 (PARK3). Clinical features relatively closely resemble those of sporadic PD. So far, however, the gene has not been identified. Interestingly, however, two independent recent reports implicate the PARK3-locus as a disease modifying locus influencing age at onset in two sib pair cohorts with PD

And a European sib pair study also identified a linkage peak in this region. A further study refined this association to a region near the sepiapterine reductase gene. Sepiapterine reductase is involved in dopamine synthesis. This finding may indicate that the SPR gene is modifying age of onset of PD.

A missense mutation in the gene for ubiquitin carboxy-terminal hydrolase L1 gene (UCHL1, PARK5), which is located on chromosome 4p has been identified in affecteds in a single family of German ancestry. To date, no other bona fide pathogenic mutations of this gene have been identified. However, a polymorphism in the UCHL1 gene has found to be associated with sporadic PD in several studies, including a large meta-analysis. Whether UCHL1 is really a rare high-penetrance PD-gene is not clear yet.

Another locus has been mapped on chromosome 1 in an Icelandic population, but the gene has not yet been identified and it is unclear, whether the locus plays a role in other populations also. Finally, a locus on chromosome 2q has been mapped in a mixed Caucasian population from the United States, but the locus has not been confirmed in European families, so again its role is still uncertain.

CONCLUSION

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.

REFERENCES


