INTRODUCTION

The field of molecular genetics has recently provided important clues to further understanding of the etiopathogenesis of idiopathic Parkinson’s disease (PD). Different mendelian forms of PD have been identified, establishing the concept of etiological heterogeneity in degenerative parkinsonisms (Table I, see over) (1-3).

The genes and loci so far identified are responsible for only a very small percentage of PD cases, and additional, as yet unknown, mendelian defects probably exist in other familial forms of the disease.

In the vast majority of sporadic cases a monogenic etiology is unlikely, and PD results from a complex interplay of several genetic as well as non-genetic factors (3,4).

However, molecular dissection of the rare monogenic forms is unraveling common pathogenic pathways that are probably involved in all the PD forms, including the sporadic ones.

PARK1 locus / α-synuclein gene

In 1996, the first monogenic form of PD was mapped to the long arm of chromosome 4 in a large Italian-American family (the “Contursi”
showing autosomal dominant transmission of the disease (5). A point mutation (G209A, leading to the substitution of Alanine by Threonine at position 53 in the protein, Ala53Thr) was later identified in one of the known genes in the linked region, encoding α-synuclein (6). This mutation (G209A) is present in the affected members of the Contursi kindred and a few smaller Greek families. Haplotype analysis has recently shown that all the "Mediterranean" families bearing the G209A mutation probably originate from a common ancestor (7).

A different point mutation (G88C, leading to Ala30Pro substitution in the protein) has subsequently been identified in a German PD family, confirming the pathogenic role of the mutations in the α-synuclein gene (8).

However, screening of large series of sporadic and familial PD cases failed to detect additional new mutations in the coding regions of the gene (9,10), and the α-synuclein locus was excluded by linkage analysis in several independent families with a dominant pattern of inheritance (11). These studies clearly indicate that mutations in the α-synuclein gene are a very rare cause of autosomal dominant PD. This form is now designated PARK1 in the online OMIM database of mendelian inheritance in man (http://www.ncbi.nlm.nih.gov/Omim).

In comparison with classical PD, the phenotype associated with the G209A mutation is characterized by an earlier onset (45 years on average, with wide variability) and a more rapid progression (12). The response to L-dopa is present, at least in the early stages, and some individuals develop dementia in addition to parkinsonism. PARK1 is associated with severe and widespread Lewy body pathology. However, the clinical features associated with the second mutation (G88C) might be more similar to classical PD (13).

PARK1 is not merely a rare monogenic PD form. Soon after the mutations in α-synuclein gene were discovered, it was shown that the wild-type α-synuclein protein is a main component (perhaps the main component) of the Lewy bodies in classical (sporadic) PD, as well as in diffuse Lewy body disease and in the Lewy body variant of Alzheimer’s disease (14,15). The aggregation of this protein could therefore be a central and primary process in the pathogenesis of the diseases associated with Lewy bodies, which have already been termed "α-synucleinopathies" (16).

However, a causal pathogenic role for α-synuclein aggregation, as well as for Lewy body formation, remains to be demonstrated. As an alternative hypothesis, α-synuclein aggregation (and Lewy body formation) could also be secondary phenomena (or even protective processes) occurring during the course of neuronal degeneration (17).

The synucleins are a family of proteins made up of about 140 aminoacids, which are highly

<table>
<thead>
<tr>
<th>OMIM ref.</th>
<th>Locus</th>
<th>Gene</th>
<th>Transmission</th>
<th>Lewy bodies ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1</td>
<td>4q21-23</td>
<td>α-synuclein</td>
<td>dominant - high penetrance</td>
<td>Yes (6)</td>
</tr>
<tr>
<td>PARK2</td>
<td>6q25.2-27</td>
<td>parkin</td>
<td>recessive</td>
<td>No (41)</td>
</tr>
<tr>
<td>PARK3</td>
<td>2p13</td>
<td>unknown</td>
<td>dominant - low penetrance</td>
<td>Yes (54)</td>
</tr>
<tr>
<td>PARK4</td>
<td>4p</td>
<td>unknown</td>
<td>dominant - high penetrance</td>
<td>Yes (59)</td>
</tr>
<tr>
<td>--------</td>
<td>4p</td>
<td>UCH-L1</td>
<td>dominant</td>
<td>unknown (56)</td>
</tr>
</tbody>
</table>

Note: PARK1, PARK2 and PARK3 are included in the OMIM database (Online Mendelian Inheritance in Man, see: http://www.ncbi.nlm.nih.gov/Omim).
conserved during evolution (for a review, see 18). Four different members are known in humans (α-synuclein, β-synuclein, γ-synuclein, synoretin) (18-20).

α-synuclein and β-synuclein share a higher degree of homology, and both are expressed in the brain, whereas γ-synuclein (persyn) is expressed predominantly in the peripheral nervous system. Interestingly, α-synuclein expression is strong in the brain regions which are highly involved in Lewy body disease (18).

Sequencing studies of α-synuclein and β-synuclein genes in PD cases have failed to reveal any mutations (21,22).

Little is known about the function of α-synuclein. It is an abundant brain protein localized in presynaptic terminals and is likely to be involved in regulation of synaptic activity (18). A central fragment of α-synuclein – non amyloid component or NAC – is the second most abundant component of amyloid plaques in Alzheimer’s disease after amyloid-beta peptide itself, and there are data that suggest a role for NAC peptide in amyloidogenesis (23-25).

Recently, the α-synuclein gene has been knocked out in mice (26). These animals are viable, fertile, and possess normal brain anatomy, including a complete set of dopaminergic neurons and synaptic terminals. However, they seem to display biochemical, neurophysiological and behavioral abnormalities that might be explained by an alteration of central dopaminergic transmission, suggesting that α-synuclein plays a regulatory role at this level (26).

Whatever the normal function of α-synuclein might be, the Ala53Thr and Ala30Pro mutations appear to cause parkinsonism in a dominant fashion. This suggests the “gain” of a new, toxic property by the mutant protein, and this new property might be completely different from the physiological one(s).

It has been shown that α-synuclein is able to form aggregates and insoluble fibrils in vitro (27). At ultrastructural level, these fibrils resemble the filaments that are found in Lewy bodies. The Ala53Thr and Ala30Pro mutations enhance the oligomerization and/or fibrillization of the protein in vitro, thus suggesting possible pathogenic mechanisms in the rare familial PD forms associated with mutations in α-synuclein (28,29).

Much less is known about the mechanisms and consequences of deposition of normal α-synuclein in sporadic PD (without mutations in the gene).

Several hypotheses have been formulated, and are presently being tested, including alterations in the control of gene expression, or changes in protein degradation (by the ubiquitin-proteasome system) (30), or aberrant interaction(s) with different specific proteins, like the recently identified synphilin-1 (31).

Moreover, α-synuclein aggregation can be induced by different oxidative stress conditions (including MPTP-induced inhibition of mitochondrial complex I in vivo, or iron- and copper-mediated oxidative stress in vitro), suggesting putative intriguing links between non-genetic factors and the α-synuclein pathogenic cascade (32-34). Finally, α-synuclein shares homology with 14-3-3 proteins, a family of cellular chaperones (proteins implicated in the control of protein folding and maturation). This suggests additional putative functions of α-synuclein and also possible pathogenic mechanisms (35).

Very recently, two studies on α-synuclein transgenic animals have been published (mice overexpressing human wild-type α-synuclein gene, and Drosophila overexpressing wild-type or mutant human α-synuclein genes) (36,37). Both these transgenic animals develop age-specific neuronal cytoplasmic inclusions immunoreactive for α-synuclein, dopaminergic neuronal deficits (mouse) and also neuronal loss (fly), and neurological deficits. These studies need to be confirmed by other independent observations. However, they strongly suggest that increased expression of α-synuclein gene or altered protein degradation are implicated in the pathogenesis of PD (36,37).

Finally, α-synuclein protein is present in the glial cytoplasmic inclusions that constitute the...
cytopathological hallmark of multiple system atrophy (MSA) (38). This suggests that MSA is also an \( \alpha \)-synucleinopathy, and that the pathogenesis of PD and MSA might be closer than previously thought (16). Sequencing the coding regions of the \( \alpha \)-synuclein gene revealed no mutations in MSA cases (39), as in the vast majority of PD familial and sporadic cases.

**PARK2 locus / parkin gene**

This monogenic form was mapped in 1997 to the long arm of chromosome 6, in a group of Japanese families with autosomal recessive parkinsonism, showing early onset, good L-dopa response, but absence of Lewy bodies (40). The following year, homozygous mutations (exon deletions) in a new gene termed “parkin” were identified in some of the Japanese families (41). The parkin gene extends over more than 500 kb, and it possesses 12 exons and very large introns. It encodes a protein made up of 465 aminoacids, sharing moderate homology with ubiquitin in the initial part, and two RING finger motifs in the terminal part, separated by an “in between ring” (IBR) domain (41,42). The presence of these domains suggests an involvement of parkin protein in the ubiquitination pathways (41,42). Recent studies also suggested that parkin is a cytoplasmic protein localised in the Golgi complex (43). The parkin gene is expressed in several brain regions (44). However, the normal function(s) of parkin, and how its deficiency results in selective neuronal degeneration, remain unknown.

The pathology of PARK2 has been studied in only very few cases so far, and it consists of neuronal loss and gliosis in the substantia nigra and locus coeruleus, with absence of Lewy bodies (45,46). Tau-positive neurofibrillary tangles have been found in one of these brains, but whether these inclusions are part of the parkin pathology or merely an associated phenomenon remains unclear (46).

Parkin disease is neither rare nor exclusive to Oriental populations (47,48). A wide variety of mutations in the parkin gene have been identified in European families with autosomal recessive early-onset parkinsonism, by the European Consortium on Genetic Susceptibility in PD (49-51). Of 73 European families with at least two affected siblings, and onset before the age of 45 in at least one sib, nearly half had parkin gene mutations (51). These mutations include several types of exon rearrangements (exon deletions as well as exon multiplications), and several point mutations (truncating or missense) in every possible combination. Interestingly, in a few families, a mutation in a single allele was detected, suggesting that further mutations remain to be discovered. These mutations are likely to reside in the non-coding regions of the gene (51). The known parkin mutations are distributed along the 12 exons of the gene, making mutational screening very laborious. Several point mutations cluster in the RING-IBR-RING region of the protein, as well as in the ubiquitin-like region, clearly delineating these as important functional domains in parkin (Fig.1) (51).

The phenotype associated with PARK2 (or “parkin disease”) is characterized by very early onset (32 years on average in Europe), very good response to L-dopa, very slow disease course and the presence of L-dopa-related motor fluctuations and dyskinesias. Cognitive and vegetative disorders are rarely observed. Additional features are the frequent presence of foot dystonia at onset, diurnal fluctuations in the severity of symptoms (sleep benefit) and brisk tendon reflexes in lower limbs (45, 51-53). However, the clinical picture can be indistinguishable from classical PD, especially in the cases with later onset (after the age of 40), and thus the diagnosis of parkin disease requires genetic testing (51).

Being a recessive trait, parkin disease can present as isolated (sporadic) early-onset PD. In the experience of the European Consortium on Genetic Susceptibility in PD, 18 out of 100 isolated PD cases with onset before the age of 45 had parkin gene mutations, and these were especially
frequent in the patients with disease onset before the age of 30 (51).

Parkin gene mutations are an important cause of autosomal recessive early-onset PD worldwide, and must be considered in the diagnostic work-up of early-onset cases. Table II (see over) summarizes the clinical features that might suggest parkin disease and might prove useful in selecting cases for genetic testing.

**PARK3 locus**

A second susceptibility locus for autosomal dominant PD was mapped, in 1998, to the short arm of chromosome 2, in four families of German-Danish ancestry (54).

The clinical characteristics of this form are very similar to classical PD, showing typical late onset (around 59 years), disease course, and presence of Lewy-body pathology. Some individuals develop dementia in addition to parkinsonism, others have isolated dementia. The pathology is consistent with a diagnosis of the limbic form of diffuse Lewy-body disease. Importantly, the estimated penetrance of the mutation is low (less than 40%). This is compatible with an involvement of this locus also in sporadic forms of PD (54). Two of the linked families (originating from southern Denmark and northern Germany) share haplotypes in the critical region, suggesting the presence of a common founder. A recent search for this founder haplotype in PD patients from northern Germany failed to identify additional carriers (55). The causative genetic defect at the PARK3 locus remains unknown.

**UCH-L1 gene mutation**

A point mutation (Ile93Met) has been identified in the gene for the enzyme ubiquitin carboxy-terminal-hydrolase-L1 (UCH-L1, which maps to chromosome 4p) in a single German family with two sibs affected by clinically diagnosed PD (56).

The mutation induces a significant reduction in the enzymatic activity measured in vitro, and it could therefore be of functional relevance, again suggesting an involvement of the ubiquitin-proteasome pathway in the pathogenesis of PD (56). However, the physiological substrate(s) of this abundant brain enzyme (which is also present in
Lewy bodies) remain(s) unknown, and subsequent screenings have failed to identify the Ile93Met or different mutations in other PD families (57,58). The pathogenic significance therefore remains uncertain, and the Ile93Met change could also be a rare neutral polymorphism.

**PARK4 locus**

In 1999, evidence emerged in support of the existence of an additional genetic locus for autosomal dominant PD on the short arm of chromosome 4 (4p), in a close but distinct region from the *UCH-L1* gene locus (59). The phenotype in this family of Iowan ancestry is characterized by early-onset PD (average 33 years), very aggressive disease course and early severe dementia and dysautonomia (60). The L-dopa response is present at the beginning of the disease and widespread Lewy bodies are found at autopsy. Interestingly, some relatives in the Iowan family show only mild postural tremor, and they share the 4p haplotype with PD relatives, giving rise to the suggestion that a single genetic defect might be associated with a phenotypical spectrum encompassing severe early-onset parkinsonism-dementia and benign, oligosymptomatic forms similar to essential tremor (59).

However, the causative gene remains unknown, and further independent evidence for linkage to 4p in other PD families has not yet been forthcoming.

**CONCLUDING REMARKS**

Four genetic loci for mendelian PD are known (PARK1/α-synuclein, PARK2/parkin, PARK3, PARK4) (Table I). In other families with PD, these loci have been excluded by linkage analysis, suggesting the existence of at least one other monogenic form of PD.

The role of these genes in the classical form of PD remains largely unknown, and different polymorphisms in these genes are currently being investigated as putative risk factors for the sporadic form of the disease (61,62).

Finally, the rare mendelian PD models (like PARK1 or PARK2) are opening up important new avenues for molecular dissection of the pathogenesis in the classical, sporadic forms of PD. Common pathogenic pathways are taking shape, involving critical proteins like α-synuclein, parkin and the ubiquitin-proteasome systems, and these discoveries hold the promise of the development of new therapeutic strategies for PD.

**NOTE ADDED TO PROOF**

The involvement of parkin in protein degradation systems as a ubiquitin-protein ligase (E3-class enzyme) has recently been shown (63). The target protein(s) for parkin-mediated ubiquitination remain(s) unknown.

**ACKNOWLEDGMENTS**

The author thanks the patients and relatives examined in recent years for their understanding and contributions, and Drs G. Meco, E. Fabrizio, N. Vanacore, N. Locuratolo, R. Marconi, M. De-
Mari, G. DeMichele, A. Filla, G. Campanella, and the members of the European Consortium on Genetic Susceptibility in PD for their collaboration.

REFERENCES

16. Spillantini MG. Parkinson’s disease, dementia with Lewy bodies and multiple system atrophy are α-synucleinopathies. Parkinsonism Relat Disord 1999;5:157-162
gamma-synuclein genes in familial autosomal dominant Parkinson’s disease. DNA Res 1998;5:401-402
an autosomal recessive form of juvenile parkinsonism to chromosome 6q25.2-27. Am J Hum Genet 1997;60:588-596


46. Mori H, Kondo T, Yokochi M et al. Pathological and biochemical studies of juvenile parkinsonism linked to chromosome 6q. Neurology 1998;51:890-892


