Parkin genetics: one model for Parkinson’s disease

Ignacio F. Mata, Paul J. Lockhart and Matthew J. Farrer*

Laboratories of Neurogenetics, Department of Neuroscience, Mayo Clinic Jacksonville, FL, USA

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The genetic epidemiology of late-onset idiopathic Parkinson’s disease (PD) and ‘parkin-proven’ parkinsonism (AR-JP) are limited. The clinical phenotype, prognosis and treatments are similar although PD is prevalent while AR-JP is rare. Molecular genetic and functional analysis suggests the E3 ubiquitin protein ligase activity of parkin, and the ubiquitin-proteosomal pathway, is central to disease pathogenesis. Herein, we compare and contrast PD and AR-JP and discuss the implications of recent data about parkin’s genomic organization, regulation and function.

INTRODUCTION

Parkinson’s disease (PD) is a prevalent age-associated progressive neurodegenerative disorder characterized by a triad of resting tremor, bradykinesia, rigidity and postural instability. Patients often display excellent initial symptomatic response to levodopa therapy, however there is no current treatment to slow PD progression (1,2). PD is a complex, multifactorial syndrome of largely unknown etiology that typically manifests after the age of 50 years. Common, co-existing features may include depression, dementia and autonomic dysfunction (3). Pathologically, the disease is associated with neuronal loss in the substantia nigra and locus ceruleus, with Lewy body inclusions in surviving cells within the brainstem (4). Definitive diagnosis is post mortem as clinically many other disorders manifest with parkinsonism without Lewy bodies, including drug-induced parkinsonism, post-encephalitic parkinsonism, progressive supranuclear palsy and spinocerebellar ataxia (types 2, 3) (5,6). A caveat is that many other diseases exhibit Lewy body pathology, including dementia with Lewy bodies, multiple system atrophy, neurodegeneration with brain iron accumulation I, Down syndrome and, occasionally, prion disorders (7–11).

Relatively little is known regarding the mechanism of PD pathogenesis, in particular the apparent susceptibility of nigral neurons to degeneration. Most cases of PD appear sporadic and perhaps are associated with a range of environmental risk factors (reviewed in 12). However, recent studies have demonstrated the importance of genetic contributions to PD and parkinsonism and may provide mechanistic detail of disease pathogenesis (reviewed in 13,14). Indeed, 10 loci have currently been identified as associated with PD (Table 1). α-synuclein (SNCA) was the first gene linked to PD, the protein being thought to play an essential role in synaptic transmission. An alanine to threonine conversion at amino acid 53 (A53T) was identified in a large Greek/Italian kindred with early-onset familial PD (15). In addition, alanine to proline (A30P) and glutamic acid to lysine (E46K) alterations in α-synuclein have been identified in rare familial PD kindreds (16,17). Dominant families with SNCA genomic multiplication, triplication and duplications, illustrate that overexpression of the wild-type protein is sufficient for disease; age at onset and disease severity is associated with copy number (18–20). Subsequently, alterations in the promoter of SNCA were shown to be associated with an increased risk of idiopathic PD (21), illustrating how results obtained from rare familial cases may have broader application. Several other genes, in addition to parkin (described below) also have been implicated in PD (Table 1). Interestingly, several of these genes are linked to the ubiquitin proteasome system (UPS), suggesting protein metabolism may play a key role in disease pathogenesis.

PARKIN-PROVEN PD: CLINICAL AND PATHOLOGICAL FEATURES

Mutations in the parkin gene (PRKN) have been associated with autosomal recessive, juvenile onset PD (22). Parkin-proven disease, caused by homozygous or compound heterozygous mutations of PRKN, has similar cardinal signs as PD, although onset is earlier (<45 years). Additional clinical features include dystonia, pronounced diurnal fluctuation and sleep benefit (23). The dystonia may be a feature of early age of onset rather than parkin mutations per se (24). Co-existing morbidity

*To whom correspondence should be addressed at: Neurogenetics, Department of Neuroscience, Mayo Clinic Jacksonville, Birdsell Bldg, Rm 206, Jacksonville, FL 32224, USA. Tel: +1 9049530158; Fax: +1 9049537370; Email: farrer.matthew@mayo.edu
may manifest as depression or psychiatric disturbance (25,26). Given that loss of parkin function is causal, there is considerable disparity in age of onset, presentation, progression and response to drug treatment, even for patients within the same sibship, with the same mutations (24,27). Treatment is similar to PD with a good response to low doses of levodopa, although dyskinesias may be early and problematic to manage given the duration of disease.

To date, there have been few reports of detailed pathology in parkin-proven disease cases (<8). Pathologically, patients with homozygous deletion of PRKN demonstrated profound neuronal loss of dopaminergic neurons in the substantia nigra and locus ceruleus (28–31). In notable contrast to idiopathic PD, Lewy bodies (LBs) were not observed, perhaps suggesting that functional parkin is a prerequisite for LB generation (32). Lewy bodies are thought to represent a mechanism whereby cells sequester damaged and toxic proteins in an inactive form. The UPS is involved in the detoxification of toxic proteins, so complete loss of parkin (a component of the UPS, see below) may be sufficient to prevent LB formation. In contrast, some patients with compound heterozygous PRKN mutations have been described with Lewy body or tau pathology (33–35). Recent studies have shown missense mutations within RING domains retain ubiquitin ligase activity (36) and confer a toxic gain of function, leading to parkin protein aggregation and aggresome formation (37,38). These results may help explain the alternate pathologies observed post mortem.

PARKIN: MOLECULAR GENETICS

Parkin is one of the largest genes in the genome. The gene has 12 exons, encoding 465 amino acids, with a super-expanded intronic structure spanning 1.3 Mb of genomic DNA (22,39) (Fig. 1). The parkin locus (PRKN), adjacent to the 6q telomere is hyper-recombining and lies within FRA6E, the third most common fragile site in tumor tissue (40,41), although the potential role of parkin in cancer is yet to be determined.

The initial parkin mutations identified were large homozygous genomic deletions, however subsequent studies also identified multiplications, small deletion/insertions and missense mutations (reviewed in 42; Table 2). PRKN semi-quantitative genomic dosage assays and sequence analysis has been performed in familial and sporadic early-onset parkinsonism (<45 years at onset) (24,26,43–47). The likelihood of PRKN mutations is inversely associated with age and may be as high as 50% in individuals with parkinsonism at age <25 years (24,47). In only a few instances has the effect of truncated or missense mutations on parkin cDNA or protein expression or activity been demonstrated and more work is required. Exonic rearrangements (deletions/duplications) tend to arise de novo, whereas the majority of missense mutations are related to a common founder (27,45). Compound heterozygous mutations, with deletion and missense mutations, explain the majority of cases with early-onset parkinsonism (<45 years at onset).

Screening the PRKN gene represents a formidable challenge (46) and thus limited genetic epidemiological data is available on the population frequency of exonic deletion/duplication and missense mutations within controls and idiopathic (late-onset) Parkinson’s disease (48). To date, community-based studies suggest that carrier status (heterozygous loss of parkin function) is not causal for early-onset disease although some promotor and coding polymorphisms have been associated with late-onset PD (24,42,44,48).

Genetic testing is now warranted for a number of heritable disorders where information on mutation status may provide a positive impact on individuals’ ‘need to know’, inform lifestyle or reproductive choices. Where testing is offered, positive and negative results are largely unequivocal and meaningful genetic counseling can be offered. Any test must consider three separate issues: interpretation, quality control and utility. For PRKN, functional and frequency data is limited and presently inadequate to inform a diagnosis. Accurate assessment is difficult given the quantitative/sequencing assays required and the size of the gene. The utility is also questionable, except to inform the reproductive choices of consanguineous families. Hence, commercial genetic testing for PRKN mutations is ill advised. As an alternative, patients might be encouraged to participate in de-identified genetic epidemiological research.

PARKIN GENOMIC ORGANIZATION

The genomic organization and transcriptional regulation of the PRKN locus may also provide insight into function. PRKN has massive introns that must be transcribed to hnRNA, then spliced to give a mature RNA message for subsequent translation (39,49). The adjacent gene, PACRG, with only five exons encoding 257 amino acids, spans a similar sized genomic distance of ~0.6 Mb (50). Both genes share a 204 bp promoter being transcribed on opposite DNA strands in opposite orientations. Given their physical proximity and common transcription factor binding motifs, some degree of co-regulation may be expected (50). However, parkin’s unique repression by N-myc is noteworthy (51), not least as it further implicates the protein in cell-cycle regulation and neuronal differentiation (52).

The organization of the PRKN/PACRG locus, conserved within vertebrates, is reminiscent of a bacterial operon and suggests functional significance (50). Interestingly, PACRG is the most recent substrate of parkin to be recognized (see below; 53). The transcriptional and post-translational regulation of the PRKN/PACRG locus suggests co-ordinate expression is biologically critical.
PARKIN, AN E3 UBQUITIN PROTEIN LIGASE

Shimura et al. (54) showed parkin was an E3 ubiquitin-protein ligase, with a Ubl domain N’ terminus, followed by two RING (Really-Interesting-New-Gene) finger domains separated by an IBR (In-Between-Ring) domain (55). The motif of two RING fingers separated by an IBR domain is common to several E3 ligases, enzymes that catalyse the conjugation of activated ubiquitin to target proteins prior to their destruction via the proteasome. The identification of parkin as a component of the ubiquitylation cycle strengthens the theory that UPS dysfunction is central to PD pathogenesis. Using different approaches many putative parkin substrates have been identified, including synphilin-1, O-glycosylated α-synuclein (α-Sp22), CHIP, Pael-R, cdc-Rel1A, cyclin E, synaptotagmin XI, SEPT5_v2 and PACRG (glup) (32,36,53,54,56–61) (Fig. 2). One may postulate parkin displays broad specificity and that many substrates accumulate in AR-JP brain with disastrous consequences for dopaminergic neurons. Indeed, accumulation of two substrates (α-Sp22 and Pael-R) has been reported in parkin-proven AR-JP brain (32,56). However, an important caveat is that many putative substrates were identified utilizing in vitro techniques and remain to be validated. In vivo up-regulation in human brain tissue from ‘parkin-proven’ disease, albeit end-stage, is an important consideration, although one might argue that the very neurons in which parkin’s substrate(s) are likely to be up-regulated are the neurons most likely to have been lost and thus substrate accumulation would be difficult to detect.

MODELS OF PARKIN FUNCTION

Cell and animal models provide a useful system to address the molecular basis of parkin dysfunction and dopaminergic cell loss. Hence, transgenic overexpression and knock-out models of PRKN have been generated to provide insight into wild-type parkin function and the mechanism of disease. Such models are critical at the present time, with the availability of human brain with ‘parkin-proven’ disease being limited. Overexpression of the wild-type parkin gene is postulated to play a protective role in the proteasomal degradation of damaged, misfolded or unwanted protein (62,63) and there is some evidence that PRKN expression is mediated by the ‘unfolded protein response’ (64) although the finding is controversial (65).

The Drosophila PRKN null-model has a shorter life-span and compromised locomotor activity, compared to its wild-type counterpart, although the former is thought to be due to peripheral muscle neurodegeneration. As yet, in the central nervous system, there is little evidence for neuronal degeneration (66). There are presently three reports of PRKN null mice. Two were created by ablation of exon 3 with consequent loss of

Figure 1. Schematic representation of the Parkin/PACRG locus. The chromosomal location is shown (left). Individual exons are shown (not to scale) with intron sizes indicated (middle). A model of the protein domain structure and location of known exon rearrangements is indicated (right).
Neither model demonstrates alteration in gross brain morphology or dopaminergic neuron loss. However, both models do exhibit nigrostriatal deficits, with subtle behavioral phenotypes which may represent a relatively early stage of the degenerative process (67, 68). The most recently described PRKN null model is the spontaneous mutant ‘Quaking’. First identified in 1964, homozygous mice are characterized by dysmyelination in the central nervous system and a quaking

<table>
<thead>
<tr>
<th>EXON</th>
<th>Variant</th>
<th>Nucleotide change</th>
<th>Amino acid change/codon</th>
<th>Reference</th>
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<td>Val115Met</td>
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<td>1362C&gt;T</td>
<td>Arg419Glu</td>
<td>GT-GG-gTA-GG (74)</td>
</tr>
</tbody>
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*This mutation affects the start codon of the parkin gene, predicting Met in codon 80 as the new start for protein translation.

The position from the ATG is shown in parenthesis (seq. ref. AB009973). The last eight variants (below line) are found at higher frequency within populations and are denoted polymorphisms.
phenotype with tonic/clonic seizures in the adult. Analysis of quaking and ENU induced quaking alleles demonstrated mutation of the quaking gene (qkI) as the cause of the dysmyelinating phenotype (69,70). The underlying genetic defect in quaking has now been defined as the deletion of a 1.17 Mb region, encompassing the promoter of qkI and the PACRG/PRKN locus, with consequent loss of PACRG and parkin protein (71). Similar to the transgenic models, alterations in dopamine metabolism and behavioral deficits have been noted, suggesting these animals might provide a model of parkin and PACRG function in neurodegeneration.

CONCLUSIONS

The rapid pace of research in the PD field has been stimulated by the identification of causative mutations in the parkin and α-synuclein genes in familial PD. Alterations in the parkin gene clearly cause a disease that overlaps clinically and pathologically with idiopathic PD. To date, no single model organism recapitulates the features of AR-JP, nor does AR-JP represent all the aspects of PD, yet each is proving immensely valuable in dissecting the normal function and interactions of parkin in the dopaminergic system. Although PD represents a complex multifactorial syndrome, genetic research continues to provide novel hypotheses and the means to test them. It is likely that there are many pathways to parkinsonism, nevertheless the genes/proteins identified, and the pathways highlighted, provide insight for future therapies.

REFERENCES


