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The effects of variants in the *PARK2 (parkin)*, *PINK1*, and *PARK7 (DJ-1)* genes along with evidence for their pathogenicity

David N Hauser, Christopher T Primiani, and Mark R Cookson

Cell Biology and Gene Expression Section, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Early onset Parkinson's disease can be caused by variants in the *PINK1*, *PARK2*, and *PARK7* genes. Since their initial discoveries, hundreds of variants have been found in these genes that are associated with a Parkinsonian phenotype. This review will briefly discuss the functions of the protein products of the three genes, then focus on the effects that disease associated variants have on these functions. We will also discuss how experimental findings can help decide whether individual variants are pathogenic or not.

Introduction

The majority of cases of Parkinson's disease (PD) are of an unknown, or sporadic, origin. However, the last twenty years have yielded many discoveries that have demonstrated that mutations in single genes can cause parkinsonism, the cluster of motor symptoms associated with PD. These gene variants can be dominantly inherited, as is the case for the *SNCA* [1,2], *LRRK2* [3,4], and *VPS35* [5,6] genes. Common variants at the *SNCA* and *LRRK2* loci also increase risk for sporadic PD [7], indicating that these two loci are pleomorphic risk loci [8]. Genetic variants can also be recessively inherited and generally cause early-onset forms of parkinsonism, sometimes with additional clinical syndromes. Variants in the *PARK2 (parkin)* [9], *PINK1* [10], *PARK7 (DJ-1)* [11] and *DNAJC6* [12,13] genes cause juvenile-onset PD while mutations in *FBXO7* [14], *PLA2G6* [15], *ATP13A2* [16], *SYNJ1* [17,18], can cause parkinsonism or more complex phenotypes. The focus of this review will be to discuss the effects of variants in the three best characterized genes that cause early-onset PD, *PARK2 (parkin)*, *PINK1*, and *PARK7 (DJ-1)*, on protein function. To provide some context for the overall interpretation of specific mutations we will first discuss some general concepts around recessive inheritance before outlining how *PINK1*, *parkin* and *DJ-1* might each be associated with mitochondrial function.

Recessive inheritance and pathogenicity

Recessive phenotypes, such as those seen in patients with early onset PD, are often the result of loss of function mutations. The best evidence that a recessive phenotype is caused by a

To whom correspondence should be addressed: Mark R. Cookson, Cell Biology and Gene Expression Section, NIA, Building 35, Room 1A116, 35 Convent Drive, MSC 3707, Bethesda, MD 20892-3707, Telephone: (301) 451-3870 (office), (301) 451-7295 (fax), Cookson@mail.nih.gov.

loss of function mutation is when large deletions of both alleles of a gene segregate with the phenotype in large families. Indeed, each of the original reports on early onset PD caused by variants in *PINK1*, *parkin*, and *DJ-1* demonstrated pathogenicity in this manner [9–11]. In such cases, the gene product is not made and the phenotype is likely caused by its absence. When large deletions and point mutations in the same gene are both shown to result in the same recessive phenotype, parsimony dictates that the point mutations are also loss of function. This guiding principle can help not only in the interpretation of the effects of known disease causing mutations, but can also be useful in determining whether disease associated mutations are truly pathogenic.

However, for many variants, determining whether they are pathogenic, that is, a causal factor for a phenotype and not merely a coincidence with a phenotype can be difficult.

Demonstration of segregation can be particularly challenging for recessive phenotypes if family sizes are too small or family history and genotypes are unavailable. This has unfortunately been the case for many of the variants in *PINK1*, *parkin*, and *DJ-1* found using targeted sequencing. Generally, in these types of studies, the three genes of interest are sequenced in a cohort of patients with early onset PD and a matched group of controls without the disease. The danger is that variants that are not pathogenic but are instead benign polymorphisms will be found by chance in some of the cases but not in controls, but only because they are too rare for their frequency to be estimated reliably. An extension to this problem is when sporadic, late onset disease cases are sequenced for a candidate gene - often one can find loss of function variants in the heterozygous state leading to ambiguity as to whether such genes act as risk factors for sporadic disease. Additionally, if *only* cases are sequenced and not controls, then the overall frequency of mutations can be underestimated in the control variants.

Resolving the pathogenic versus benign variants can therefore often be difficult. However, functional assays can be used to either strengthen or weaken support for the pathogenicity of nominated variants. For instance, if loss of function variants have been demonstrated to be pathogenic, then newly nominated variants should also then be loss of function. Therefore, if a nominated variant behaves as a loss of function variant in biochemical or cellular, assays then the evidence for pathogenicity of that variant is bolstered. If a nominated variant is shown to behave like the wild type in these types of assays then it is less likely to be pathogenic. The major limitation in such assays is that a degree of *a priori* knowledge of the biology of the gene product is required for face validity.

The aim of this review is not to detail every variant of *PINK1*, *parkin*, and *DJ-1* found to associate with early onset PD, which is largely available online (The Parkinson's Disease Mutation Database [19] (<http://www.molgen.vib-ua.be/PDMutDB>)) and summarized in Figure 1. Instead, our goal is to review the effects of genetic variants on the structure and function of *PINK1*, *parkin*, and *DJ-1* that have been examined in experimental systems. To first provide some biological context to the effects of specific mutations, we will provide an overall outline of the *PINK1/parkin* pathway.

Recessive parkinsonism genes and mitochondria

The cellular functions of PINK1, parkin, and DJ-1 have been reviewed extensively [20–24] in recent years but will be briefly covered here as this is important in interpreting the effects of specific mutations. Early work identified that parkin is a member of the E3 ligase family that mediate the addition of ubiquitin to target proteins. PINK1 has a clear kinase domain and a mitochondrial targeting sequence. Therefore, while both parkin and PINK1 were immediately identifiable as enzymes, they were not obviously linked to each other.

Studies in *Drosophila* models established that PINK1 and parkin operate in a single genetic pathway that maintains functional mitochondria [25–27]. Critical features of these models are that (1) loss of function PINK1 and parkin mutations phenocopy each other with flight defects, male sterility and dopaminergic cell loss (2) parkin can rescue PINK1 but not the other way around, suggesting that PINK1 is upstream of parkin. The phenotypes in *Drosophila* are likely consequential of mitochondrial dysfunction, with strong morphological evidence of damage to mitochondria preceding other phenotypic events. Endogenous parkin likely mediates the disposal of mitochondria during *Drosophila* aging [28]

Subsequent to these studies, it was shown that parkin is recruited to depolarized mitochondria in a PINK1 dependent manner and promotes their removal via mitophagy in mammalian cells [29–31]. Mechanistically, PINK1 phosphorylates both ubiquitin and the ubiquitin-like domain of parkin to activate parkin and promote its recruitment to mitochondria [32–34]. The significance of this PINK1/parkin mediated pathway of mitophagy in the mammalian brain has been somewhat controversial because mouse models have failed to demonstrate that this pathway is active *in vivo* [35,36]. In spite of this, several groups have demonstrated that exogenous parkin localizes to mitochondria in cultured primary neurons in response to various stressors such as protonophores [37,38], reactive oxygen species [39], and glutamate [40]. The subsequent parkin mediated degradation of mitochondria in cultured neurons has been reported in some [39,40], but not all [41], studies. In summary, the PINK1/parkin pathway of mitophagy has been clearly delineated in cultured cells but its occurrence in the mammalian brain remains controversial.

It is important to note that in *Drosophila* parkin can function in the absence of PINK1 whereas in mammalian cells PINK1 is absolutely required for parkin recruitment. Quite why these species differences are seen is not yet clear; it is not likely that *Drosophila* and mammalian parkin are different proteins as the human gene can rescue the loss of function fly mutation. It is also of interest that PINK1 and parkin mutations express themselves differently in different species – the phenotypes in flies are stronger than the parkinsonism in humans while in mice there are only very subtle phenotypes. How these observations should be interpreted is not yet clear, but they are *caveats* that show that there is likely still important information about PINK1 and parkin to be gathered.

PINK1 and parkin have also been demonstrated to control several other aspects of mitochondrial quality control (reviewed in [22,42,43]). For instance, the two proteins have been shown to control the movement of mitochondria along axons by controlling the degradation of the adaptor protein Miro in both fly and mammalian neurons [44,45]. PINK1 and parkin are also known to affect mitochondrial fission and fusion in both mammalian

neurons [46] and *Drosophila* muscle [47]. These effects are likely due to the ability of parkin to ubiquitinate the mitofusin proteins and regulate their degradation [48–51]. The promotion of mitochondrial fission and the stalling of mitochondrial trafficking seem to be important precursors to mitophagy and it is therefore likely that PINK1 and parkin have the ability to co-ordinate several slightly different functions. As a further example of this principle, PINK1 and parkin have recently been shown to control the formation of mitochondria-derived vesicles that contain damaged proteins and are subsequently trafficked to the lysosome for degradation [52].

Parkin has also been shown to have functions at mitochondria that may be somewhat independent of mitochondrial quality control. Early studies in *Drosophila* indicated that apoptosis was increased in the flight muscles of *parkin* null flies [53,54]. It has been subsequently demonstrated that parkin prevents the release of cytochrome c from mitochondria by ubiquitinating the pro-apoptotic protein Bax which sequesters it away from mitochondria [55,56]. The effects of parkin on Bax and apoptosis can be observed in primary neurons and do not appear to be mutually exclusive to parkin's effects on mitophagy [57]. Additionally, parkin has been proposed to control the turnover of ZNF746, a putative transcription factor upstream of the transcriptional coactivator, PGC-1 α that is known to regulate mitochondrial biogenesis [58]. Both of these examples are tied to parkin's function as an E3 protein-ubiquitin ligase.

DJ-1 is a member of a superfamily of proteins with conserved homologues from prokaryotes to mammals, or about three billion years of evolution [59,60]. Although the functions of many members of this superfamily have been determined, the precise function of human DJ-1 remains elusive. Before it was linked to PD, it had been established that DJ-1 is sensitive to oxidative modification by reactive oxygen species [61,62]. It was later shown that cysteine 106 (C106) is particularly sensitive to oxidation to sulfinic acid [63–65]. The oxidation of C106 promotes an interaction between DJ-1 and mitochondria, and the ability of C106 to oxidize is critical for DJ-1 to exert protection against various sources of oxidative stress at the mitochondria of cells in culture [63,66]. *In vivo*, DJ-1 knockout mice are more sensitive to the mitochondrial toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [67]. DJ-1 knockout mice also exhibit increased levels of oxidative stress in the mitochondria of dopamine neurons located in the substantia nigra under basal conditions [68]. Although, how DJ-1 confers anti-oxidant protection has not yet been fully elucidated, the available data suggests an important role that is likely relevant to mitochondria as these organelles represent a major source of ROS in cells.

The functions of PINK1, parkin, and DJ-1 therefore intersect at the mitochondria, but DJ-1 is not necessary for the recruitment of parkin to depolarized mitochondria [31,38,69,70] and is able to protect cells from the complex I inhibitor rotenone in cells with and without PINK1 [71]. Together these data show that DJ-1 is not upstream of parkin [31,38,69,70] or PINK1 [71]. There are, however, relationships between DJ-1 and PINK1/parkin. For instance, both PINK1 and parkin can rescue the mitochondrial fragmentation caused by the loss of DJ-1 in primary neurons and immortalized cells [71,72]. In flies, overexpression of DJ-1 can rescue the defects caused by the loss of PINK1 but not the loss of parkin [73]. DJ-1 also rescues the hypersensitivity of dopaminergic neurons in PINK1 knockout mice to

MPTP when overexpressed in the substantia nigra [74]. Thus, it seems that DJ-1 operates in a pathway parallel to PINK1/parkin to maintain mitochondrial integrity during times of stress. Collectively, these biological observations frame how we can assay functions for PINK1, parkin and DJ-1. Next, we will discuss each specific gene product in turn.

PARK2 (parkin)

After linkage mapping to the long arm of chromosome 6 (6q25.2-q27) in Japanese families [75,76], deletions in a gene from this region were shown to cause autosomal recessive parkinsonism which was cloned and named *PARK2* [9]. The *PARK2* gene spans a 1.38 Mb region of chromosome 6 and has 12 exons that code for a 465 amino acid protein named parkin. Since this initial discovery, parkinsonism associated deletions have been reported for all exons [19], clearly showing that loss of parkin function causes disease.

Many missense mutations have also been observed in early onset PD, but these have varying strength of evidence for pathogenicity. To understand which are more likely to be causal, it is important to discuss the biochemistry of parkin. Parkin contains an N-terminal ubiquitin-like (Ubl) domain followed by four zinc finger domains (Figure 2.A), is a member of the RING-In Between-RING family of E3 ligases, and functions as a hybrid of both RING-type and HECT-type E3 ligases [77]. The RING1 domain binds E2 ubiquitin-conjugating enzymes while the RING2 domain contains an active site cysteine that forms an intermediate thioester with ubiquitin prior to transfer to the substrate [77]. The structure of parkin has recently been solved by several groups [78–81] (Figure 3). The overall structure of the protein is reminiscent of a coiled snake [82], with the RING2 domain folded underneath the RING0 domain. This formation effectively hides the active site contained in the RING2 domain, thereby holding parkin in an auto-inhibited state [79,81]. Parkin shifts from this auto-inhibited state to an active state when it interacts with ubiquitin that has been phosphorylated by PINK1 [32–34], although the structural basis of this activation is not fully understood.

Disease associated missense mutations in parkin map to all of the protein's domains [19,80]. Important insights into the mechanism and likelihood of true pathogenic variants can be inferred from the recently reported structures. For instance, the catalytic cysteine residue (C431) that is essential for the formation of a thioester intermediate with ubiquitin is located in the RING2 domain. Several disease associated variants, including the C431F mutation [83], have been found in the RING2 domain suggesting that these mutants cause a loss of function by preventing thioester intermediate formation. Mutations in the RING2 domain could have similar effects either altering the reactivity of C431 or by altering the domain's structure. Indeed, the T415N [84–86], G429E [87], and G430D [88,89] variants all fold normally but are inactive or weakly active in autoubiquitination assays, and NMR analysis shows that these variants cause chemical shifts that alter the reactivity of C431 [78]. In contrast to variants in RING2, variants in RING1 might disrupt either domain folding or the binding of the E2 enzyme. One such variant, T240M/R [90,91], is predicted to alter the structure of the E2 binding interface [79] which may explain why the T240R variant of parkin does not bind the E2 enzyme UbcH7 [92].

Cellular assays have also helped to discover how parkinsonism associated variants affect parkin. In HeLa cells, exogenous parkin translocates from the cytosol to mitochondria within a few hours of mitochondrial membrane depolarization with the protonophore CCCP [29]. If the duration of the CCCP treatment is extended to a day, then mitochondria are removed from the cells in a parkin dependent manner [29]. These observations have been widely replicated in transformed cells, and can be used to measure the effects of missense mutations in parkin. At least two groups have used recruitment of parkin or clearance of mitochondria to examine the effects of a range of parkin mutations [30,93]. Both studies found that mutations of cysteine residues important for RING domain zinc binding that are known to disrupt protein solubility [94,95] such as the C212Y [96], C298G [97], and C441R [98] variants, as well as the K211N [99] mutant in the RING0 domain, inactivate the protein in both assays [30,93]. Geisler et al. found that the P37L, R42P, R256C, G328E, and R334C variants, all of which have been found in patients with early onset PD [85,97,100–102], behaved like the wild type protein in both assays [93]. In contrast, Narendra et al. found the R42P mutant to have diminished activity in both assays. It should be noted that of the tested variants, R42P has fairly strong genetic evidence for pathogenicity in that three brothers with homozygous R42P alleles all had early onset parkinsonism but neither heterozygous parent was affected [101]. One of the most interesting finds from both papers is that the R275W [85] variant is recruited to the mitochondria following CCCP treatment at near wild type levels but cannot promote their autophagy [30,93]. Thus, parkin translocation to depolarized mitochondria and the subsequent parkin-dependent removal of them can be differentially effected by mutations in parkin.

PINK1

In 2001, a novel locus for early onset PD was identified in a large Italian kindred with four affected members on chromosome 1p35-36 [103]. Three years later, PTEN-induced kinase 1 (official gene name: *PINK1*) was identified as the gene that caused disease in this and two other families [10]. *PINK1* has eight exons, spans 18 Kb and codes for a protein of 581 amino acids. The original three families described had either a missense mutation (G309D) or a single base mutation in exon 7 that results in the truncation of the final 145 amino acids of the protein [10]. Subsequently, a patient has been identified with one *PINK1* allele completely deleted and the other allele bearing a splice site mutation that causes the generation of several aberrant mRNAs [104]. Another study also identified homozygous deletions of *PINK1* exons 4-8 in three affected siblings from a consanguineous family [105]. One mutant that is relatively common in the Philippines, L347P, has enhanced protein turnover in cells, thus leading to loss of function because there is no functional protein [106]. Thus, it is likely that all variants in *PINK1* that cause early onset PD are loss of function.

PINK1 is a serine/threonine kinase with a mitochondrial targeting sequence at the N-terminus followed by a transmembrane domain (Figure 2.B). GST-fusion constructs containing the kinase domain of human PINK1 have demonstrated that the protein is capable of autophosphorylation *in vitro* [106–108], and that the G309D variant of PINK1 has diminished activity in this assay [106,108]. However, human PINK1 kinase activity is much lower than several insect homologues of PINK1, with the *Tribolium castaneum* homologue

(tcPINK1) having the highest activity [109]. When TcPINK1 harbors kinase domain mutations associated with early onset PD such as A217D [110], E240K [111], H271Q [112], L347P [112], L369P [113], G386A [113], C388R [114], G409V [113], P416R [115], E417G [112], G440E [116], and L489P [111], *in vitro* kinase activity on a peptide substrate is completely abolished [109]. It is unclear whether most of these twelve variants are actually pathogenic or not, but the effect they have on *in vitro* kinase activity would suggest that they are indeed pathogenic. The C125G [113] variant, which lies outside the kinase domain, and the G309D variant in the kinase domain has some residual activity [109]. Recently, tcPINK1 was shown to phosphorylate ubiquitin *in vitro* [32,33], although the effects of disease associated variants in this assay have not yet been reported.

Variants in PINK1 have also been examined using cellular assays. As described above, the recruitment of parkin to mitochondria in HeLa cells is dependent on PINK1 [30,93]. Using this assay, Narendra et al. found that parkin is not recruited to depolarized mitochondria in PINK1 knockout MEF cells expressing the A168P [117] or H271Q [112] variants [30]. They also found that the L347P mutant is unstable in cells, consistent with previous reports [30,106]. Interestingly, parkin recruitment was not completely abolished in cells expressing the G409D variant [30], which is consistent with this variant possessing some *in vitro* kinase activity [106,109]. In contrast to the other variants analyzed, the G411S variant recruited parkin at the same level as wild type PINK1 [30]. It is highly unlikely that the G411S variant is pathogenic since it has only been found in the heterozygous state in PD patients [89,118–120], and has been found in a control sample [86],

Several other variants of PINK1 associated with disease have also been found to behave like the wild type protein in cellular assays. A screen of PINK1 variants measuring mitochondrial localization of YFP-parkin that was co-transfected with PINK1 in HeLa cells treated with CCCP has also been performed [121]. In this experiment, nine of the seventeen disease associated variants had no effect on the ability of PINK1 to promote parkin translocation to mitochondria [121]. The variants that did not act as loss of function were P52L [86], R68P [117], C92F [117], R147H [122], A168P [117], A427E [86], N521T [10], D525N [117], and C575R [119].

In another set of experiments, fusing PINK1 to the outer mitochondrial membrane anchor of OPA3 was found to stabilize the protein on mitochondria and promote parkin recruitment to them without the need for depolarization [123]. One surprising result found using this assay was that some variants of PINK1 are temperature sensitive and able to support parkin recruitment at ambient temperature but not at 37°C [123]. A panel of PINK1 variants that were annotated as pathogenic, pathogenic nature unclear, and not pathogenic in PDMutDB were assayed using this temperature sensitive assay. Interestingly, several of the variants annotated as unclear such as Q126P [124], G395V [125], P399L [126], R407Q [127], and G409V [113] were unable to recruit parkin at 37°C but did promote some recruitment at 22°C, albeit not as much as wild type PINK1 [123]. Seven other variants of unknown pathogenic nature were found to recruit parkin at 37°C as well as wild type PINK1, while five did not at either temperature [123].

Taken together, these results imply that a proportion of missense variants in PINK1 that have been reported to be associated with early onset PD are not pathogenic. However, there is surprisingly little overlap between the variants studied in the different assays by different groups, suggesting that there may be technical differences between assays. It is also a possibility that PINK1 has other functions and that the assays currently available do not measure, which leaves open the possibility that variants that behave like wild type in parkin recruitment assays may perturb some other function of PINK1. Thus, a consensus cannot yet be made regarding the pathogenicity of a substantial number of PINK1 variants.

PARK7 (DJ-1)

A locus on chromosome 1p, distinct from PINK1 (PARK6), associated with autosomal recessive PD was identified in 2001 [128,129]. Subsequently, the *DJ-1* gene (official gene name: *PARK7*) was demonstrated to be the affected gene at this locus in two families [11]. In one affected family from the Netherlands, the phenotype is caused by homozygous deletion of exons 1-5, while then phenotype is caused by a homozygous missense mutation (L166P) in an Italian family [11]. DJ-1 protein can not be detected in lymphoblasts from exon 1-5 deletion patients showing that loss of DJ-1 function is important for disease [130]. In total, twenty-three variants in *DJ-1* have been associated with early onset PD.

Much of what is known about DJ-1 stems from the high resolution structures solved using x-ray crystallography of the protein soon after it was associated with PD [131–134] (Figure 4). DJ-1 belongs to a diverse group of proteins known as DJ-1/PfpI superfamily which are characterized by having a highly conserved α/β fold [134]. The tertiary structure of DJ-1 shows similarities to the *P. furiosus* cysteine protease, PfpI [135], and an *E. coli* molecular chaperone, heat shock protein 31 (Hsp31) [136]. However, PfpI exists as a hexamer while DJ-1 is a dimer in both the crystal and living cells [131,137]. The formation of the DJ-1 dimer is mediated by the two most C-terminal alpha helices (alpha G and alpha H), of which alpha H is unique to DJ-1 and not present in PfpI [131].

L166, which is mutated to proline in the Italian kindred with early onset PD, is part of the penultimate alpha helix [11]. Insertion of a proline into Alpha G is results in significant structural alterations to the helix and thereby disrupts dimerization [131]. CD spectroscopy of recombinant L166P DJ-1 suggests that it exists in a random coil structure, consistent with the thermal denaturation profile of recombinant L166P DJ-1 [138]. A small amount of recombinant L166P DJ-1 migrates as an apparent monomer on size exclusion chromatography columns, but most elutes in the void volume, further suggesting unfolding [138]. Collectively, these studies show that L166P has a strong structural effect in vitro.

Lymphoblasts from patients carrying the homozygous L166P mutation have greatly decreased levels of detectable DJ-1 protein [130]. In cultured cells L166P mutation leads to enhanced degradation through the ubiquitin-proteasome system [138–140] and possibly non-proteasomal pathways [141] without loss of mRNA expression [139,140]. Thus, the L166P mutant disrupts DJ-1 folding, which is recognized by the cell and the mutant protein is rapidly degraded.

Several other homozygous variants in *DJ-1* have been found in humans with early onset PD including L10P [142], M26I [143], E64D [144], G78G [143], P158del [145], E163K [146], and a promoter region duplication (g.168_185dup) [146]. These mutations are incredibly rare, collectively having been found in a homozygous state in only eight humans. Being so rare, whether or not these mutations are actually pathogenic is unclear but both structural and cell biology studies have examined how they affect protein stability and function.

Recombinant M26I DJ-1 is able to form a dimer, but the stability of the protein is slightly decreased relative to wild type [147]. M26I DJ-1 is also more prone to aggregation *in vitro* than wild type DJ-1 [148]. Most groups have found that M26I DJ-1 can form dimers in cells [140,149,150], although one group has found that it impaired dimer formation using a bimolecular fluorescence complementation assay [151]. The authors of the latter study suggested that their findings were due to the low amounts of M26I protein present in their cells [151]. Low steady state levels of M26I DJ-1 have also been found by other groups [149,150,152,153], but others have found that M26I DJ-1 is stable when expressed in other cell lines using different expression constructs [140,154]. Overall, if the M26I variant destabilizes DJ-1 then it does so in a much more subtle way than L166P.

The effects of several other mutations in DJ-1 have been studied to varying degrees. The E64D mutant crystallizes as a dimer and appears similar to the wild type protein when assessed using several biophysical measurements [141,144,148,155]. However, the levels of this variant in fibroblasts from the patient bearing the homozygous mutation are decreased via an unknown mechanism [144,156]. Other variants such as E163K reduce the stability of the protein *in vitro*, but crystallize normally as a dimer [147]. Lastly, the L10P and P158del variants are unstable and impair dimer formation when expressed in cells [157]. Altogether, there is very little information about most of the DJ-1 variants apart from L166P and M26I. Although these variants are extremely rare, they merit further study, which could yield valuable insights into the true function of DJ-1.

Several putative functions for DJ-1 and its homologues have been reported including chaperone activity [158], protease activity [159], glyoxalase activity [160], and RNA-binding [161]. However, purified human DJ-1 exhibits no protease activity in several *in vitro* assays [131,158]. Protease activity is unlikely to be a DJ-1 function because His126, which has been proposed to part of the catalytic dyad essential for protease activity, is not well conserved in flies and prokaryotes [162] and is oriented incorrectly in the human protein [131]. Hsp31 proteins, which are members of the DJ-1 superfamily, have been shown to have glutathione-independent glyoxalase activities in multiple species [163–165]. Human DJ-1 is a member of a separate clade from the Hsp31 proteins and lacks a histidine residue that is important for Hsp31 glyoxalase activity [163,165]. Accordingly, low glyoxalase activities have been reported for human DJ-1 [163] and its close homologues such as *S. pombe* DJ-1 [163,165] and *E. coli* YajL [160]. Although, one study has found that human DJ-1 also has *in vitro* glyoxalase activity when measured at temperatures above physiological relevance for humans (45°C) [160]. Thus, the physiological relevance of any glyoxalase activity and other putative functions for human DJ-1 and its close homologues is unclear.

Finally, the fact that mutations in DJ-1 are loss of function should be considered when designing and evaluating experiments to assay the function of DJ-1. Over-expressing loss of function DJ-1 mutants in the presence of wild type endogenous protein is likely to result in data that are difficult to interpret. Rather, wild type and loss of function mutants should be re-expressed in a DJ-1 null background and compared to interpret the how the mutations affect any possible functions of the wild type protein.

Conclusions

This year will mark the seventeenth anniversary of the discovery that variants in the *parkin* gene caused early onset PD. The years since have seen the discoveries that early onset PD can also be caused by variants in several other genes. These events are milestones in our understanding of familial PD, and research on the protein products of these genes have bolstered important theories about the pathogenesis of parkinsonism that mitochondrial dysfunction and oxidative stress seem to play key roles in the disease mechanisms that underlie PD.

There is, however, much that is still not understood about these proteins. Future research should strive to meet several key goals. First, the effects of all known variants in *parkin* and *PINK1* on the ability of these two proteins to identify and mediate the disposal of damaged mitochondria should be determined. Preliminary screens of some mutants have been performed, but a complete screen of all variants could yield new insights into their effects and, importantly, their plausibility of being pathogenic. Secondly, now that the structure of parkin has been determined, an effort should be made to discover what effects disease associated variants have on it. Third, solving the structure of PINK1 would be extremely helpful in understanding of the effects of disease associated variants in this protein. Finally, the function of DJ-1 remains unknown. Determining what this highly conserved and abundant protein does and developing robust assays of this function, as is the case for PINK1/parkin, may help understand why DJ-1 is in a somewhat parallel pathway for recessive parkinsonism.

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Figure 1. Quantity and type of variants identified to date in PINK1, PARK2, and PARK7
The number of individual variants reported in the literature to date and their classification by the PD Mutation Database (PDMutDB) are shown in the graph.

Figure 2. Diagrams of parkin, PINK1, and DJ-1 proteins

A. Schematic diagram of Parkin representing its RING domains; UBL: ubiquitin-like domain, IBR: in-between-ring domain. B. PINK1 domains; MTS: mitochondrial targeting sequence, TM: transmembrane domain. C. DJ-1 .

Figure 3. Stereo view of the parkin ribbon structure

Colors correspond to the protein domain colors diagramed in Figure 2A. The eight navy colored spheres represent Zn^{2+} that are stabilized by the RING domains and the catalytically active cysteine, C431, is depicted in red. Protein graphics were created using UCSF Chimera (PDB 4k95)

Figure 4. Structure of the DJ-1 dimer

A. DJ-1 dimer ribbon (top) and surface structure (bottom) looking into the barrel of the first alpha helix (α A) with C106 and L166 colored. B. View of the dimer interface in relation to the L166 variant on the penultimate alpha-helix (α G). C. View of the DJ-1 monomer with the surface colored according to hydrophobicity (red indicates more hydrophobic areas). The protein is oriented so that the hydrophobic dimer interface faces the viewer. D. View of the DJ-1 dimer oriented exactly as it is shown in (B) with the surface colored according to hydrophobicity and the location of L166 labelled. Protein graphics were created using UCSF Chimera (PDB 1p5f)