Genetic Mouse Models of Parkinsonism: Strengths and Limitations

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Summary: Parkinson's disease (PD) is a progressive neurodegenerative disorder. Patients with PD display a combination of motor symptoms including resting tremor, rigidity, bradykinesia, and postural instability that worsen over time. These motor symptoms are related to the progressive loss of dopamine neurons in the substantia nigra pars compacta. PD patients also suffer from nonmotor symptoms that may precede the cardinal motor symptoms and that are likely related to pathology in other brain regions. Traditional toxin models of PD have focused on the nigrostriatal pathway and the loss of dopamine neurons in this region, and these models have been important in our understanding of PD and in the development of symptomatic treatments for the disease. However, they are limited in that they do not reproduce the full pathology and progression seen in PD, thus creating a need for better models. The recent discovery of specific genes causing familial forms of PD has contributed to the development of novel genetic mouse models of PD. This review discusses the validity, benefits, and limitations of these new models. **Key Words:** α-Synuclein, parkin, DJ-1, Nurr1, Pitx3, mice.

Parkinson's disease (PD) is one of the most common neurodegenerative disorders. PD is primarily characterized by the loss of dopamine (DA) neurons in the substantia nigra and the development of proteinacious neuronal inclusions called Lewy bodies. Typically at the time of diagnosis, patients suffer from motor impairments, including bradykinesia, tremor, rigidity, and postural instability that worsen as the disease progresses. These motor symptoms are associated with the progressive loss of nigrostriatal DA neurons. In addition, patients also suffer from nonmotor symptoms such as olfactory impairments, gastrointestinal dysfunction, depression, sleep disturbances, and cognitive impairments that are likely related to alterations in brain regions other than the substantia nigra and may precede the cardinal motor symptoms.[1–3] Although most cases of PD are sporadic, the recent discovery of specific mutations in genes that cause familial forms of PD has led to a new approach in the study of PD. Several genes including α-synuclein, parkin, DJ-1, UCHL1, Pink1, and most recently LRRK2[4–13] have been implicated in familial PD (Table 1). In addition, the discovery of genes essential for the development of DA neurons has provided clues to the susceptibility of DA neurons in PD. Both approaches have led to the generation of novel animal models: genetic mouse models of PD.

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α-SYNUCLEIN MICE

α-Synuclein (PARK 1) was the first gene to be linked with PD when two missense mutations (A30P and A53T) were identified in familial PD.[6,8] This discovery led to the identification of α-synuclein as being one of the major components of Lewy bodies and glial cytoplasmic inclusions in sporadic PD, dementia with Lewy bodies, and multiple system atrophy. Based on this common cytopathological hallmark, these movement disorders are now classified as synucleinopathies.[14] Functionally, α-synuclein (PARK 1) is a 140-amino acid presynaptic protein notably involved in vesicle handling and neurotransmitter release.[15,16]

The identification of a third mutation (E46K)[17] and more recently of genomic multiplications (duplication or triplication) of a locus containing the α-synuclein gene in several PD families[9,18,19] further highlights the importance of endogenous α-synuclein in the disease. Even though there is so far no direct relationship between sporadic PD and α-synuclein expression, the existence of several polymorphisms in the promoter of the α-synuclein gene

TABLE 1. *Identified Genes Associated with Familial Parkinson's Disease*

Park	Locus	Gene	Function	References
Park 1	4q21–22	α-Synuclein	Presynaptic	8
Park 2	6q25–27	Parkin	E3 ubiquitin ligase	5
Park 5	4p14	UCH-L1	Ubiquitin recycling enzyme	10
Park 6	1p25–36	PINK1	Mitochondrial	11
Park 7	1p36	DJ1	Oxidative stress response	4
Park 8	12p11.2q13.1	LRRK2	Kinase	12

suggests that expression levels might be a risk factor.[20–22] Given the widespread occurrence of α-synuclein aggregation in sporadic synucleinopathies, and the pathogenic role of increased endogenous α-synuclein in familial forms of PD due to genomic multiplications, α-synuclein has been the focus of an increasing number of investigations aimed at elucidating its pathophysiological significance.

Several transgenic mouse lines including α-synuclein knockout or overexpression of human α-synuclein (wild-type, A30P, A53T or A30P+A53T) have been generated. α-Synuclein knockout mice show reduced rearing activity in the open field, decreased DA content in the striatum,[23] and a decrease of the reserve pool of vesicles in the hippocampus.[15] More recently, a double knockout for α- and β-synuclein shows a similar reduction in striatal DA.[24] These results confirm *in vivo* the fine regulatory role of α-synuclein in synaptic plasticity and vesicle maintenance. Interestingly, several lines of α-synuclein null mice have a complete[25] or partial[26,27] resistance to the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Overexpression of human α-synuclein in mice results in variable neuropathological and behavioral phenotypes, and expression levels can be estimated to range from 0.5–30 fold compared with endogenous α-synuclein.[28–36] Interestingly, the most pronounced phenotypes are consistently observed in the highest expressing lines, regardless of the promoter used to drive the transgene. This supports the emerging idea that expression levels can play a significant role in the disease progression and manifestation.[9]

Several lines of α-synuclein overexpressing mice show pathology in areas such as the spinal cord and in neuromuscular junctions[29–32] and no pathology in the nigrostriatal pathway. Of the many different α-synuclein overexpressing mice generated, only a few show alterations in the nigrostriatal DA system (Tables 2 and 3).[33–35] Although none of the transgenic lines initially reported a loss in dopaminergic neurons, mice overexpressing the doubly mutated A30P + A53T α-synuclein under the TH promoter present a progressive reduction of striatal dopamine transporter (DAT) density and reduced DA and metabolite levels.[33] Subsequently, it was shown that these mice do have a progressive loss of Nissl-stained neurons in the substantia nigra at 8.5 and 19 months, but the number of TH-positive neurons remains stable.[37] Compatible with this pathology, these mice show age-related motor impairments including decreased locomotor activity and increased righting time on an inverted screen.[33] These mice also show a reduced response to the dopaminergic agonists apomorphine and amphetamine.[33,37] Similarly, mice that overexpress wild-type human α-synuclein under the PDGFβ promoter show reduced TH activity and DA content in the striatum and behaviorally they have impaired performance on the rotarod.[35]

In mice overexpressing human wild-type α-synuclein under the Thy1 promoter, α-synuclein overexpression is widespread throughout the brain including cortex, substantia nigra, and olfactory areas[34] and proteinase K-resistant inclusions and increased microglia are found in the substantia nigra (Hutson, C. B., P.-O. Fernagut, and M.-F. Chesselet, unpublished observations). In these mice, the substantia nigra is vulnerable to subtoxic doses of MPTP,[38] and they show a wide range of progressive sensorimotor impairments including impaired performance on a challenging beam, impairments orienting on a pole, decreased spontaneous activity, altered response to sensory stimuli, and impairments in fine motor skills.[39] These impairments progressively worsen with age and can be detected as early as 2 months.[39] These mice have a small but significant decrease in DAT and vesicular monoamine transporter (VMAT) binding in the striatum at 8 months (Fernagut, P.-O., and M.-F. Chesselet, unpublished observations). Similar to the A30P + A53T α-synuclein mice, the Thy1 α-synuclein mice show an abnormal behavioral response to dopaminergic agonists including L-DOPA, apomorphine, and amphetamine (Fleming, S. M., J. Salcedo, C. B. Hutson, E. Rockenstein, E. Masliah, M. S. Levine, and M.-F. Chesselet, submitted). Notably, they show a marked decrease in amphetamine-induced stereotypies, but increases in sensitivity to low doses of apomorphine. In addition, these mice also show impairments in olfaction and gastrointestinal function; both impairments are associated with the nonmotor symptoms seen in PD and provide further support for α-synuclein's involvement in PD (Fleming, S. M., L. Wang, Y. Tache, and M.-F. Chesselet, unpublished observations).

TABLE 2. *Nigrostriatal Pathology in Genetic Mouse Models of PD*

Mouse	Mutation	DA Pathology	References
α-Synuclein overexpressor	A53T + A30P: TH promoter	↓ Striatal DA, DOPAC, HVA, ↓ striatal DAT, ↓ SNc neurons	33, 37
	Human wild type: PDGFβ promoter	↓ TH activity in striatum, ↓ striatal DA content	35
	Human wild type: Thy1 promoter	↑ SNc Vulnerability to MPTP, ↓ striatal DAT and VMAT	34, 38; Fernagut, P.-O., and M.-F. Chesselet, unpublished observations
Parkin knockout	Exon 3 deletion	↑ DA; ↓ amphetamine induced DA release; ↓ DAT; inhibition of glutamate transmission	51
	Exon 3 deletion	↑ Extracellular DA; reduced synaptic excitability in striatal medium spiny neurons; mitochondrial dysfunction	52, 55
	Exon 7 deletion	↓ NE in olfactory bulb and spinal cord; ↓ TH-positive neurons in LC	53
	Exon 2 deletion	None detected	54
	Spontaneous (Quaking mouse)	↑ DA metabolism; ↑ D2 DA receptor striatal membranes	60
DJ-1 knockout	Exon 2	↓ Evoked DA overflow in striatum, ↓ sensitivity on SNc neurons to D2 agonist, lack of LTD in striatal medium spiny neurons	74
DJ-1 knockout	Exon 1 stop codon	↑ SNc vulnerability to MPTP	75
Nurr1 heterozygote	Reduced Nurr1	↓ Striatal DA content, ↓ SNc neurons, ↑ SNc vulnerability to MPTP	80, 82
Pitx3-aphakia	Loss of Pitx3	↓ Striatal DA content, ↓ SNc neurons, L-DOPA-mediated induction of c-*fos*	61, 83, 84

SNc = substantia nigra pars compacta; NE = norepinephrine; TH = tyrosine hydroxylase; LC = locus coeruleus; LTD = long-term depression; HVA = homovanillic acid.

Despite the lack of a massive loss of DA nigrostriatal neurons, the α-synuclein overexpressing mice with alterations in the nigrostriatal system are useful models of PD because they have mutations that are similar to those found in familial PD, thus giving them construct validity. These mice also have face validity because they have pathology and behavioral impairments reminiscent of the pathology and symptoms observed in the disease. Furthermore, similar to PD, the α-synuclein mice with nigrostriatal alterations, have impairments that worsen with age. They will be particularly useful to study the etiology of PD and in understanding the interaction between environmental and genetic factors in relation to PD. In addition, the benefit of a model without nigrostriatal DA loss is that the early stages of the disease, before the loss of nigrostriatal DA neurons, can be studied. Several of these models have a modest loss of DAT and VMAT in the striatum, indicating early stages of DA pathology.[40,41] In addition, the abnormal response to DA agonists observed in these mice[33,37] further suggests that they may represent a model of early dopaminergic dysfunction. Moreover, some lines of α-synuclein overexpressing mice show neuronal death outside the substantia nigra and/or premature death[29,30] making them suitable for testing neuroprotective strategies (Table 4).

PARKIN KNOCKOUT MICE

Soon after the discovery of α-synuclein mutations associated with familial PD, it was shown that loss of function mutations in the parkin gene are linked to familial autosomal recessive juvenile PD. The typical age of onset in autosomal recessive juvenile PD is less than 50 years, and patients suffer from motor symptoms similar to idiopathic PD including rigidity, resting tremor, and bradykinesia. Patients respond to L-DOPA therapy; however, they develop L-DOPA-induced dyskinesias sooner than patients with idiopathic PD.[42] Pathologically, patients also have a degeneration of nigrostriatal DA neurons but most do not develop Lewy bodies.[43–46]

Parkin (PARK 2) is a 465-amino acid protein that acts functionally as an E3 ligase and is involved in the ubiquitination of proteins for degradation by the proteasome.[47–49] It is thought that mutations causing a loss of parkin function can lead to the abnormal accumulation of parkin substrates. These substrates include glycosylated α-synuclein,[48] synphilin-1,[50] Pael-R,[47] and CDCrel-1[49] and may play an important role in the development of the disease. Knocking out parkin function in mice has been

TABLE 3. *Behavioral Anomalies in Genetic Mouse Models of PD*

Mouse	Mutation	Behavioral Impairments	Effect of DA Agonists	References
α-Synuclein overexpresor	A53T + A30P: TH promoter	Locomotor activity, righting on an inverted screen	Reduced locomotor response to amphetamine and apomorphine	33, 37
	Human wild type: PDGFβ promoter	Rotarod	NA	35
	Human wild type: Thy1 promoter	Challenging beam, pole test, inverted grid, spontaneous activity, response to sensory stimuli, and fine motor skills (bin cotton use).	Increased sensitivity to apomorphine, reduced response to amphetamine	39; Fleming, S. M., J. Salcedo, C. B. Hutson, E. Rockenstein, E. Masliah, M. S. Levine, and M.-F. Chesselet, submitted
Parkin knockout	Exon 3 deletion	Locomotor activity, alternation in T-maze	Reduced response to amphetamine	51
	Exon 3 deletion	Challenging beam, response to sensory stimuli	Decreased grooming response to intrastriatal DA	52; Fleming, S. M., N. T. Maidment, and M.-F. Chesselet, unpublished observations
	Exon 7 deletion	Startle response	NA	53
	Exon 2 deletion	None detected in tests used	No difference in locomotor activity after amphetamine	54
	Spontaneous (Quaking mouse)	Locomotor activity	Enhanced response to SKF 38393, Reduced response to LY 171555	60
DJ-1 knockout	Exon 2	Locomotor activity	Reduced locomotor activity response to quinpirole	74
DJ-1 knockout	Exon 1 stop codon	None detected in tests used	Reduced locomotor activity response to amphetamine	75
Nurr1 heterozygote	Reduced Nurr1	Rotarod, locomotor activity	Enhanced locomotor activity after methamphetamine	82
Pitx3-aphakia	Loss of Pitx3	Challenging beam, pole test, and spontaneous activity	Reversal with L-DOPA	61

NA = not assessed.

accomplished by deletion of exon 3,[51,52] exon 7,[53] or exon 2[54] in the parkin gene (Tables 2 and 3).

Deletion of exon 3 in parkin results in the absence of the parkin protein. In mice expressing this mutation,[51,52] there is no overt loss of TH-positive neurons in the substantia nigra or their projections to the striatum; however, more subtle nigrostriatal DA alterations have been shown. Parkin knockout mice with an exon 3 deletion have increased extracellular striatal DA, reduced synaptic excitability in striatal medium spiny neurons, and progressive sensorimotor impairments including increased errors while traversing a challenging beam and alterations in their response to sensory stimuli.[52] In addition, these parkin knockout mice show a reduction in weight gain, reduced mitochondrial respiration, reduced antioxidant capacity, and increased oxidative damage in the ventral midbrain.[55] Similarly, in a separate study, another line of mice with an exon 3 deletion of parkin

TABLE 4. *Construct, Face, and Predictive Validity in Genetic Mouse Models of Parkinson's Disease*

Mouse	Construct (Etiology)	Face (Symptoms and Pathology)	Predictive (Response to Symptomatic Treatment)	Predictive (Testing Neuroprotection Strategies)
α-Synuclein overexpressor	Good	Good	Poor	Good
Parkin knockout	Good	Weak	Poor	Good
DJ-1 knockout	Good	Weak	Poor	Good
Nurr1 heterozygote	Weak	Good	NA	Good
Pitx3-aphakia	Poor	Good	Good	Poor

NA = not assessed.

show an inhibition of amphetamine-induced DA release in fetal midbrain cultures, inhibition of glutamate transmission in the hippocampus, reduced DAT protein in the striatum, and motor and cognitive deficits including reduced locomotor activity and decreased spontaneous alternation in the T-maze.[51]

Targeted disruption of exon 7 in parkin also results in the loss of parkin function.[53] Parkin knockout mice with an exon 7 deletion appear to show a different phenotype compared with exon 3 deleted mice; however, they have not been examined in the same comprehensive battery of behavioral tests. Exon 7 parkin knockout mice show reduced acoustic startle response and a loss of TH-containing neurons in the locus coeruleus, which likely occurs during development. In addition, these mice show reduced norepinephrine levels in olfactory bulb and spinal cord. Similar to exon 3 deleted mice, there is no loss of nigrostriatal DA neurons. This is the only parkin knockout mouse to show neuronal loss in the locus coeruleus, and degeneration in this area is a feature of PD.[56]

Similar to both exon 3 and exon 7 deletions, deletion of parkin exon 2 results in the loss of function of parkin.[54] These mice show no loss of nigrostriatal DA neurons; however, unlike the previously mentioned parkin knockout mice, exon 2-deleted mice show no neuronal loss in the locus coeruleus, no alterations in catecholamine levels and no detectable behavioral impairments.[54] Although different tests have been used, and DA release or synaptic function have not been measured in these mice, it is possible that either the type of mutation or the genetic background of the mice could contribute to differences in phenotypes between these various lines of mice.

The *quaking viable* mouse has been known for sometime,[57] it was only recently discovered that these mice also have deletions in parkin and parkin coregulated genes.[58,59] Similar to exon 3-deleted parkin knockout mice, quaking mice have alterations within the nigrostriatal DA system.[60] Compared with controls, quaking mice have increased levels of the DA metabolites DOPAC and homovanillic acid in the striatum and olfactory tubercle, respectively, and increased DA D2 receptors. In addition to their quaking phenotype, behaviorally, these mice also show a greater sensitivity to DA D1 receptor agonists and are less sensitive to DA D2 agonist treatment.[60]

Despite the lack of accumulation of parkin substrates in parkin knockout mice, their DA phenotype makes them useful in the study of PD. Like α-synuclein mice, parkin knockout mice have a mutation similar to that found in familial PD, which gives them construct validity. This model has face validity because parkin knockout mice show similar behavioral impairments compared to both α-synuclein overexpressing mice and mice with known loss of nigrostriatal DA neurons (Pitx3-aphakia mice).[39,61] Both exon 3 and exon 7 deleted parkin knockout mice may be most beneficial in the understanding of the etiology and the early stages of PD, and in the interaction between environmental and genetic factors associated with the disease. The model is limited by the absence of loss of nigrostriatal DA neurons. In addition, the behavioral phenotype of these mice is modest compared to α-synuclein overexpressing, and Pitx3-aphakia mice (see below). However, they show chronic changes in DA release and synaptic dysfunction in the striatum, indicating that they are useful to identify the early effects of a PD-causing mutation *in vivo* (Table 4).

DJ-1 KNOCKOUT MICE

Deletion (exons 1–5) or point mutation in DJ-1 have been recently linked to autosomal recessive early onset parkinsonism in two families[4] and other mutations in the DJ-1 gene have been identified in several cases of early onset parkinsonism.[62–64] However, DJ-1 mutations appear to be less frequent than parkin mutations.[64] DJ-1 is only rarely detected in Lewy bodies.[65] DJ-1 (PARK 7; 189-amino acid protein) appears to be a multifunctional protein as it was initially identified as an oncogene product,[66] then as a regulatory subunit of a RNA binding protein,[67] and has been implicated in the cellular response to oxidative stress.[68,69] Given the multiple functions of DJ-1, especially its involvement in oxidative stress, it may play a role in the pathophysiology of PD. Indeed, deficits in complex 1, decreased antioxidant defenses, and oxidative stress have all been linked to sporadic PD.[70] Downregulation or knockout of endogenous DJ-1 *in vitro* increases the vulnerability to oxidative stress and proteasome inhibition.[71,72] DJ-1 also has a redox-dependant chaperone function and inhibits the aggregation of α-synuclein.[73]

Recently, mice with a targeted deletion of exon 2,[74] or insertion of a premature stop codon in exon 1[75] of the DJ-1 gene have been generated (Tables 2 and 3). Similar to α-synuclein and parkin knockout mice, DJ-1 knockout mice do not lose nigrostriatal DA neurons.[74,75] Deletion of DJ-1 exon two has subtle effects on the nigrostriatal pathway. Although TH activity, mRNA expression, and dopamine and its metabolites were unaltered in these mice, evoked dopamine release on striatal slices was clearly reduced, most likely as a consequence of increased reuptake. Nigral neurons from DJ-1 exon 2 mutant mice were less responsive to D2 autoreceptor stimulation and were not more vulnerable to paraquat intoxication. Behaviorally, mutant mice showed decreased locomotor activity. Loss of DJ-1 function through insertion of a premature stop codon in exon 1 did not affect the number dopaminergic neurons or their fiber density in the striatum. Reduced locomotor activity was revealed only upon methamphetamine or MPTP chal-

lenge. *In vitro*, primary cortical neurons from DJ-1 mutant mice were more sensitive to oxidative stress, and mesencephalic neurons were more sensitive to rotenone.[75] DJ-1 mutant mice also had an increased sensitivity to MPTP, both at the level of dopaminergic neuronal loss and TH loss in the striatum. This was rescued by restoring DJ-1 expression in mutant mice, further indicating a role for DJ-1 in oxidative stress response.[75] Similar to α-synuclein overexpressing and parkin knockout mice, DJ-1 knockout mice have good construct validity. This model should be of interest to explore the function of DJ-1 *in vivo* and its potential relevance to sporadic PD (Table 4).

NURR1 AND PITX3-APHAKIA MICE

Nurr1 is a member of the nuclear receptor superfamily[76] and is involved in the differentiation and development of nigrostriatal DA neurons. Mutations in Nurr1 alter transcription of the gene that encodes tyrosine hydroxylase[77] and transcription of the dopamine transporter[78] and suggests that alterations in Nurr1 may cause chronic DA alterations that could increase susceptibility to PD. Indeed, it has been shown that mutations in Nurr1 may be associated with familial PD.[79] Nurr1 is essential for the development of ventral mesencephalon DA neurons as homozygous Nurr1 knockout mice do not develop DA neurons in the substantia nigra and die soon after birth.[80] In contrast, heterozygous Nurr1 knockout mice do survive and show normal levels of nigrostriatal DA neurons, but they do exhibit a vulnerability to neurotoxins such as MPTP.[81] More recently, it was shown that, as Nurr1 heterozygous mice age, they develop a decrease in DA levels in the striatum, decreased DA nigrostriatal neurons, and decreased locomotor activity and rotarod performance.[82] These data indicate a progressive DA phenotype in these mice that bears some resemblance to that found in α-synuclein overexpressing and mutant mice.[33,35,37,39]

Similar to Nurr1, Pitx3, a homeobox transcription factor, is involved in the molecular development of nigrostriatal DA neurons.[83,84] Pitx3-deficient mice (Pitx3-aphakia mice)[85] have a loss of nigrostriatal DA neurons and a 90% reduction in DA content in the striatum, whereas DA neurons in the ventral tegmental area are spared.[84] Behaviorally, Pitx3-aphakia mice display sensorimotor impairments on a challenging beam, deficits orienting on a pole, and a decrease in spontaneous rearing. All of these impairments are reversed with L-DOPA treatment.[61]

Both Nurr1 heterozygous and Pitx3-aphakia mice are good models for PD because they have the characteristic loss of nigrostriatal DA neurons. In Nurr1 heterozygous mice, this is even progressive, as in patients (Tables 2 and 3), giving them good face validity. In addition, Pitx3-aphakia mice have predictive validity for symptomatic treatment in that L-DOPA reverses several of the sensorimotor impairments in these mice. However, these models are limited because they do not reproduce the broad pathology seen in PD and only show pathology in the nigrostriatal pathway (Table 4). Furthermore, it is unclear that the mechanism involved in the cell loss operates in PD. Therefore, their usefulness to test neuroprotective strategies is limited.

Although the DA phenotypes of the α-synuclein, parkin knockout, and DJ-1 knockout mice are not as profound as the Nurr1 heterozygous and Pitx3-aphakia mice, they may provide insight into the early stages of the disease. A model of the early stages of PD is an important tool because it allows further investigation into the early events that may lead to the characteristic loss of nigrostriatal DA neurons. Furthermore, animals with known genetic mutations associated with PD can be used to study the mechanisms of the disease and identify therapeutic targets. The Nurr1 and Pitx3-aphakia mice provide good models to study the later stages of the disease and DA neuronal loss that may lead to the development of better symptomatic treatments for PD. Although no perfect animal model of PD exists yet, the mice discussed model complementary aspects of PD and will be important in understanding the etiology and progression of PD.

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