Published in final edited form as:
Parkinsonism Relat Disord. 2012 January ; 18(0 1): S180–S182. doi:10.1016/S1353-8020(11)70056-6.

Genetic mouse models for understanding LRRK2 biology, pathology and pre-clinical application

Zhenyu Yue*
Department of Neurology and Neuroscience, Mount Sinai School of Medicine, New York, NY 10029, USA

SUMMARY

Missense mutations in Leucine-Rich Repeat kinase 2 (LRRK2) are the most common cause of inherited Parkinson's disease (PD). Elucidation of LRRK2 biology and pathophysiology is central to the development of therapeutic intervention. Our group and others have developed a number of genetic mouse models of LRRK2 utilizing different genetic approaches. These models exhibit certain PD-related pathologies (e.g. impaired dopamine transmission and tauopathies) and abnormal motor functions, providing valuable insight into potential LRRK2-mediated pathogenesis of PD. However, not surprisingly they lack of substantial neuropathology and clinical syndromes of PD. Ongoing investigation of these models has begun to shed light on LRRK2 cellular functions and pathogenic pathways and is expected to assist the identification and validation of PD drug targets. This report summarizes the recent findings in our genetic LRRK2 models and discusses their utility in understanding much needed knowledge regarding early stage (pre-symptomatic) disease progression, drug target identification, and potential application in chemical screening focused on inhibitors of kinase activity of LRRK2.

Keywords

Genetic mouse models; LRRK2; Parkinson's disease

1. Introduction

Genetic identification of causative PD genes has been fruitful and provided compelling evidence for inherited form of PD. Recent cloning of PARK8 (LRRK2) is particularly promising as LRRK2 mutations contribute to the most common familial form and some sporadic cases of PD [1,2]. LRRK2 encodes a complex protein (285 kD) containing kinase and GTPase activities, which are apparently altered by several familial LRRK2 mutations [3]. G2019S mutation, located in kinase domain of LRRK2, is the most prevalent mutation, present in more than 85% of PD patients carrying LRRK2 mutations. The G2019S was also identified in 1% of sporadic PD cases, linking LRRK2 pathogenic pathway to the idiopathic PD [4,5].

The majority of LRRK2 pathogenic mutations cause clinically typical PD. Biochemical analysis suggests that some, but not all, PD-linked mutations in LRRK2 cause increased kinase activity [3,6], which is correlated with enhanced neuronal toxicity [6–8]. The

*Correspondence: Tel.: +1 212 241 3155 (office); fax: +1 212 241 3869. zhenyu.yue@mssm.edu (Z. Yue).

Conflict of interests
The author has no conflicts of interest to declare.

previous study in invertebrate models implicates LRRK2 in diverse cellular functions in vesicular trafficking, neurite outgrowth, cytoskeletal regulation and translational control [9].

Modeling PD in genetically modified rodents is critical to the understanding of the pathogenesis of PD and developing therapeutic strategy. Recently several groups including ours have reported the generation of LRRK2 genetic mouse models. All LRRK2 genetic models are viable, fertile, and no gross abnormality in development and differentiation has been observed. There is lack of substantial neurodegenerative process and clinical syndromes of PD. However, common PD-related pathologies were observed in these genetic models. In this report, I summarize the characterization of our LRRK2 models and discuss their potential in understanding much needed knowledge regarding early stage (pre-symptomatic) disease progression, identifying drug targets, and exploring the potential in aiding compound screening for the inhibitors of kinase activity of LRRK2.

2. BAC transgenic models of LRRK2

We used bacterial artificial chromosome (BAC)-mediated transgenesis to develop mouse models expressing PD-linked mutations of LRRK2. The BAC transgenesis permits precise temporal and spatial expression pattern of transgene under the control of endogenous promoter [10]. BAC transgenesis is particularly suitable for the modeling of neurodegenerative diseases with dominant disease transmission. Our group developed two BAC transgenic models expressing mouse LRRK2 wildtype (LRRK2-Wt) and G2019S mutant [11]. Analysis of the two BAC models showed similar expression patterns and levels in the CNS, offering a unique opportunity to “tease out” G2019S-specific disease effect from phenotypes caused by LRRK2-Wt overexpression. The characterization revealed distinct effect of LRRK2-Wt vs. G2019S on striatal dopamine transmission (cyclic voltametry) and consequent motor function: LRRK2-Wt mice have elevated striatal dopamine release with unaltered DA uptake or tissue content, and accordingly, they are hyperactive and show enhanced performance in motor function tests; by contrast, LRRK2-G2019S mice show an age-dependent decrease in striatal DA content (~25%), as well as decreased striatal DA release and uptake. However, LRRK2-G2019S mice display no motor function deficits up to 12 months [11]. Interestingly, a number of LRRK2-G2019S mice (10–15%) developed abnormal motor behavior around 20 months of age (Yue lab, unpublished result). Furthermore, we reported that LRRK2-Wt and G2019S overexpression have distinct effects on phospho-tau levels in certain brain regions, suggesting that LRRK2-Wt may regulate phospho-Tau homeostasis that is impaired in G2019S mutant [11].

What are the implications of the above results? First, despite the lack of degeneration of dopamine neurons and α-synuclein inclusions in the above models, the BAC mice expressing G2019S mutant exhibit pathological features that are highly relevant to PD: impairment of striatal dopamine transmission and striatal dopamine content, aberrant regulation of phospho-tau levels (compared to LRRK2-Wt) and late onset motor abnormality. Considering the enhancement of dopamine transmission and motor performance caused by overexpression of wildtype LRRK2, we propose that at system level LRRK2 plays an important role in regulating striatal dopamine transmission and consequent motor function control; the G2019S pathogenic mutation disrupts LRRK2-mediated dopamine transmission, which is linked to motor function deficits. Second, the results suggest a tie between LRRK2 and tau, in agreement with the observation in a subset of LRRK2 mutation PD patients showing tauopathies [2,12–14]. Third, not surprisingly, some discrepancies of certain phenotypes exist in our BAC models and others. It is conceivable that different BAC constructs, host strain genetic background, expression levels of transgenes, can account for the variability of the phenotypes in these BAC models [15].

3. LRRK2 cellular functions and pathogenic pathway

3.1. Role of LRRK2 in neurotransmission and regulation of synaptic vesicles

A common observation emerging from the studies of multiple LRRK2 models is the impairment of dopamine transmission in G2019S and R1441C/G BAC mice. LRRK2 mutations may also affect other neurotransmitter system. Furthermore, the impaired neurotransmission cannot be necessarily secondary to degenerating presynaptic terminals, as no obvious axonal abnormalities were noticed in the BAC G2019S mice [11]. Therefore, the PD mutations can trigger an initial defect in neuronal/axonal functions in the absence of physical denervation of presynaptic terminals. Previous evidence that LRRK2 function is linked to the regulation of synaptic vesicle protein localization or synaptic vesicle trafficking is compatible with this notion. Therefore, impairment of synaptic vesicle function caused by G2019S or R1441C/G could be the underlying mechanism for presynaptic dysfunction.

3.2. Role of LRRK2 in Tau and cytoskeletal dynamics

Our study provides a support for the link of LRRK2 and Tau in the same pathway. Indeed, recent genome-wide association studies (GWAS) showed surprisingly that MAPT (genetic term for tau) is a risk factor for sporadic PD [16], therefore placing tau in the pathogenic cascade of PD in addition to other neurological disorders with tau pathology such as Alzheimer's disease and frontotemporal dementia. It also suggests that these diseases may in part share the pathogenic mechanism involved in tau dysfunction. A line of evidence has indicated that LRRK2 can interact and/or phosphorylate proteins involved in microtubule and actin dynamics. This may explain the axonal pathology and presynaptic abnormality in several LRRK2 genetic models [15].

3.3. Potential link of LRRK2 and α-synuclein

A previous study showed that LRRK2 expression levels are critical for the development of α-synuclein-induced neuropathology in mice, suggesting that LRRK2 acts upstream and regulates α-synuclein in disease pathway [17]. Interesting, a relevant report in LRRK2 knockout mice shows that endogenous LRRK2 appears to be important to suppress abnormal accumulation of α-synuclein as well as accompanied inflammatory response in kidney [18]. Given the current knowledge about α-synuclein function, one can speculate that in the CNS LRRK2 and α-synuclein are functionally related and cause neuropathology perhaps through the following pathways: (1) interfere with synaptic vesicle functions by directly engaging vesicle-associated proteins and membrane trafficking; and/or (2) impair microtubule and actin dynamics by modulating cytoskeletal protein biochemical properties at presynaptical terminals.

3.4. Phosphorylation, 14-3-3 binding of LRRK2, and impairment of phosphorylation/14-3-3 binding in LRRK2 PD mutants

We identified multiple phosphorylation sites at N-terminus of LRRK2 including S910, S912, S935 and S973 in LRRK2 protein isolated from brain, suggesting that LRRK2 is extensively phosphorylated *in vivo*. Our study showed that 14-3-3 proteins bind LRRK2 and this binding depends on phosphorylation of S935. The 14-3-3 binding of LRRK2, with little effect on dimer formation of LRRK2, confers protection of the phosphorylation status of S935. We also find that that protein kinase A (PKA), but not LRRK2 kinase itself, can cause the phosphorylation of LRRK2 at S935 *in vitro* and in cell culture, suggesting that PKA is a potential upstream kinase that regulates LRRK2 function. Further, our study indicates that the common PD-related mutations of LRRK2, R1441G, Y1699C and G2019S, decrease homeostatic phosphorylation levels of S935 and impair 14-3-3 binding of LRRK2 [19]. The result suggests that 14-3-3 is an important regulator of LRRK2-mediated cellular functions,

and that the impairment of phosphorylation/14-3-3 binding of LRRK2 caused by the common familial PD-related mutations provides a potential pathogenic mechanism of LRRK2-linked PD.

4. How do we evaluate LRRK2 BAC models lacking overt neurodegeneration

The lack of the substantial PD neuropathology and clinical features of the PD syndrome raises the question how we evaluate the LRRK2 models for basic and clinical research of PD. The late onset and age-dependent penetrance of the G2019S may explain the lack of neurodegeneration in our LRRK2 BAC models with maximal 2–3 year in life span. A number of functional imaging studies show abnormal striatal dopamine system in both symptomatic G2019S patients, which is indistinguishable from idiopathogenic PD cases [20], and asymptomatic G2019S individuals, who might be in the course of developing PD symptoms. This result demonstrates that the striatal dopamine abnormality occurs preceding the frank motor function deficits in G2019S PD patients and perhaps loss of the dopamine neurons. Therefore, the LRRK2 models recapitulate the specific disease events, and may represent an early pathological alteration before the loss of dopamine neurons. Therefore, we propose that these models can be best used to identify the molecular events at early disease stage that trigger signaling cascade and are causative to the ultimate manifestation of motor deficits and neurodegeneration. In addition, they should be valuable for delineating the sequence of pathological events that are responsible for the slow progression of the disease. Finally, PD is a motor as well as non-motor disease. LRRK2 is expressed in broad brain regions that are in charge of more than just motor functions. In addition, LRRK2 patients also exhibit non-motor symptoms similar to sporadic PD [4]. It is worth mentioning that our G2019S models showed certain abnormal non-motor behavior, which was associated with pre-motor episode in PD. Future experiments should investigate the molecular and cellular basis of this observation and may shed light on the pathogenic mechanism for the non-motor components of PD.

5. Application of the LRRK2 models in pre-clinical research

The toxic gain-of-function in LRRK2 kinase activity has been a dominant hypothesis, and therefore, inhibition of kinase activity is being pursued as the primary therapeutic strategy for LRRK2 mutant PD. How can the BAC LRRK2 models aid the target validation and inhibitor screening?

With increasing number of LRRK2 inhibitors identified, a suitable mouse model(s) is highly desirable for the functional validation of their efficacy *in vivo*. Although the rodent models mediated by HSV or adenovirus delivery of LRRK2 may provide promising neurodegenerative models, the expansion of the study for thorough screening and validation of inhibitors may be difficult due to overall high variability of the results in this type of study. Despite the lack of the prominent PD neuropathology, multiple lines of LRRK2 BAC transgenics share some PD relevant pathological features and can assist to establish potential endpoints in evaluating the LRRK2 inhibitors.

First, efficacy of small chemical-mediated inhibition of LRRK2 activity in the CNS should be demonstrated. This can be assessed by evaluation of autophosphorylation of LRRK2 and phosphorylation of LRRK2 substrates via phospho-specific antibody detection. In our BAC LRRK2-Wt and G2019S mice, we showed that the kinase activity of the brain G2019S protein is 2–3-fold higher than that of wildtype [11]. These models are potentially useful to test the idea that suppression of enhanced kinase activity in G2019S (without a complete block of wildtype LRRK2 kinase activity) is correlated with the reversal of the observed

neuropathology in G2019S mice. Second, we propose the premise of impaired striatal dopamine transmission (and levels) and tau pathology, the two common disease-related features in LRRK2 genetic models, as primary neuropathology endpoints, and the motor function deficits in the BAC mice as potential behavioral endpoint. Third, more than one model should be tested in order to draw a meaningful conclusion.

6. Future perspective

Continuous exploration of the existing and incoming new LRRK2 models will provide critical information for drug target validation and novel endpoint identification in the development of therapeutic strategies. As LRRK2 kinase/GTPase activity is a highly "druggable" target, therapeutic strategies targeting aberrant LRRK2 activity hold a great promise for the treatment of PD. Probe of the specific disease feature and stage with LRRK2 inhibitors in available LRRK2 genetic models should be highly feasible, and is expected to advance our knowledge regarding how LRRK2 kinase/GTPase is involved in disease progression and move the field of PD research forward.

Acknowledgments

Funding support: NIH/NINDS grants NS060809–01, NS072359–01 and Michael J. Fox Foundation for Parkinson's Research.

References

1. Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 2004; 44:595–600. [PubMed: 15541308]
2. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron. 2004; 44:601–7. [PubMed: 15541309]
3. West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, et al. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. Proc Natl Acad Sci U S A. 2005; 102:16842–7. [PubMed: 16269541]
4. Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case–control study. Lancet Neurol. 2008; 7:583–90. [PubMed: 18539534]
5. Correia Guedes L, Ferreira JJ, Rosa MM, Coelho M, Bonifati V, Sampaio C. Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. Parkinsonism Relat Disord. 2010; 16:237–42. [PubMed: 19945904]
6. Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, Ross CA. Kinase activity of mutant LRRK2 mediates neuronal toxicity. Nat Neurosci. 2006; 9:1231–3. [PubMed: 16980962]
7. Ito G, Okai T, Fujino G, Takeda K, Ichijo H, Katada T, Iwatsubo T. GTP binding is essential to the protein kinase activity of LRRK2, a causative gene product for familial Parkinson's disease. Biochemistry. 2007; 46:1380–8. [PubMed: 17260967]
8. West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, et al. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet. 2007; 16:223–32. [PubMed: 17200152]
9. Yue Z. LRRK2 in Parkinson's disease: in vivo models and approaches for understanding pathogenic roles. FEBS J. 2009; 276:6445–54. [PubMed: 19804414]
10. Heintz N. BAC to the future: the use of bac transgenic mice for neuroscience research. Nat Rev Neurosci. 2001; 2:861–70. [PubMed: 11733793]
11. Li X, Patel JC, Wang J, Avshalumov MV, Nicholson C, Buxbaum JD, et al. Enhanced striatal dopamine transmission and motor performance with LRRK2 overexpression in mice is eliminated

by familial Parkinson's disease mutation G2019S. J Neurosci. 2010; 30:1788–97. [PubMed: 20130188]

12. Rajput A, Dickson DW, Robinson CA, Ross OA, Dachsel JC, Lincoln SJ, et al. Parkinsonism, Lrrk2 G2019S, and tau neuropathology. Neurology. 2006; 67:1506–8. [PubMed: 17060589]
13. Cookson MR. The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. Nat Rev Neurosci. 2010; 11:791–7. [PubMed: 21088684]
14. Wszolek ZK, Pfeiffer RF, Tsuboi Y, Uitti RJ, McComb RD, Stoessl AJ, et al. Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. Neurology. 2004; 62:1619–22. [PubMed: 15136696]
15. Yue Z, Lachenmayer ML. Genetic LRRK2 models of Parkinson's disease: Dissecting the pathogenic pathway and exploring clinical applications. Mov Disord.
16. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, Paisan-Ruiz C, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009; 41:1308–12. [PubMed: 19915575]
17. Lin X, Parisiadou L, Gu XL, Wang L, Shim H, Sun L, et al. Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. Neuron. 2009; 64:807–27. [PubMed: 20064389]
18. Tong Y, Yamaguchi H, Giaime E, Boyle S, Kopan R, Kelleher RJ 3rd, et al. Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. Proc Natl Acad Sci U S A. 2010; 107:9879–84. [PubMed: 20457918]
19. Li X, Wang QJ, Pan N, Lee S, Zhao Y, Chait BT, et al. Phosphorylation-dependent 14-3-3 binding to LRRK2 is impaired by common mutations of familial Parkinson's disease. PLoS One. 2011; 6:e17153. [PubMed: 21390248]
20. Goldstein DS, Imrich R, Peckham E, Holmes C, Lopez G, Crews C, et al. Neurocirculatory and nigrostriatal abnormalities in Parkinson disease from LRRK2 mutation. Neurology. 2007; 69:1580–4. [PubMed: 17625107]