Parkinson disease and leucine-rich repeat kinase 2 gene mutation: abridged secondary publication

SL Ho *, PWL Ho, DCW Siu

KEY MESSAGES

1. Sporadic Parkinson disease (PD) involves a complex interaction of three major risk factors: genetic susceptibility, environmental toxicity, and ageing. We developed a new experimental protocol using a mouse model that carries a specific mutation at the same genetic location in parallel with the humans. We administered twice weekly oral doses of a naturally occurring pesticide (rotenone) over half the lifespan of the mouse to mimic chronic exposure to environmental toxicity. Ageing is inherent in the course of experiments. We found brain abnormalities and locomotor deficits in the new model are more faithful of the human PD than other existing models.
2. Although our LRRK2 knockin mice do not show obvious disease phenotype, they had greater reduction in striatal dopamine uptake, with locomotor deficits that were slower to recover than wild-type mice after reserpine injection (dopaminergic vesicle uptake blocker). These indicate that the mutant mice are more susceptible to striatal synaptic dysfunction even at young age, supporting its relevance in PD.
3. We cross-bred a new colony of mutant LRRK2 mice with fluorescent dopaminergic cells. This enables us to study, isolate, or image live dopaminergic neurons that degenerate in PD with greater ease and clarity. Our gene profiling studies showed significant differences in expression of several genes in nigrostriatal dopaminergic neurons of aged mutant mice.
4. The role of LRRK2 in phosphorylation of Rab GTPases proteins was explored using our LRRK2 mutant mice, including live mouse embryonic fibroblasts. This has important implications for the development of new treatments for PD such as LRRK2 inhibitors.
5. There is a lack of treatment modalities that can modify the progression of PD. Existing treatments relieve symptoms without altering its progression. It is still unclear how PD develops and progresses. Our model can help elucidate its complex disease processes and test therapeutic agents on these processes.

Hong Kong Med J 2020;26(Suppl 8):S22-6
HMRF project number: 01120976

SL Ho, PWL Ho, DCW Siu

Division of Neurology, Department of Medicine, The University of Hong Kong

* Principal applicant and corresponding author: slho@hku.hk

Introduction

Parkinson disease (PD) is a common neurodegenerative disease involving loss of dopaminergic neurons in substantia nigra par compacta, with nerve terminals projecting to the striatum. Progressive striatal dopamine depletion and synaptic dysfunction result in locomotor deficits. The disease process involves a complex interaction of ageing, genetic susceptibility, and environmental factors.

PD is rare before age 50 years and its occurrence increases substantially with ageing. Whole genome studies have linked several genes to sporadic PD. Farmers chronically exposed to environmental factors (eg pesticides) are more likely to develop PD. A naturally occurring pesticide, rotenone, is used in experimental models of PD. Current therapies of PD fail to modify disease progression. Hence, developing an appropriate experimental model involving these aetiological factors is crucial to explore the disease processes and develop therapeutic strategies.

Almost all existing experimental models of PD either use high doses of toxins given over a short period or use genetic models that either overexpress (transgenic) or have absent (knockout) gene expression. Although high acute doses of toxins can cause acute parkinsonian features, it does not reflect the situation in human PD that progresses gradually. Hence, chronic small doses of a toxin may better reflect the environmental toxicity.

Leucine-rich repeat kinase 2 (LRRK2) mutations are the commonest genetic risk in familial and sporadic PD. LRRK2-associated PD demonstrates similar clinical features as sporadic PD, suggesting parallel disease mechanisms. Although existing LRRK2 transgenic and knockout mice have parkinsonian features, they have abnormal LRRK2 expression which do not reflect human mutant

LRRK2 carriers who have similar LRRK2 expression levels as those without the mutation (wild-type [WT]). The key feature of our knockin mice is that the desired LRRK2^{R1441G} mutant gene is integrated into a known intended locus in the genome. This is important because the mutated LRRK2 gene will achieve biological or natural expression patterns and levels as in WT. In contrast, the desired gene in the transgenic model is randomly integrated in the host genome under its own artificial promoter, leading to abnormally high expression levels with more robust phenotype. Although the transgenic model is useful in exploring gene function, the tradeoff is that transgenic model does not faithfully reflect the human disease. The gene expression pattern might be totally different from the native protein. For instance, overexpression of the LRRK2 gene in all brain regions in transgenic mice could lead to ectopic expression (ie, abnormally high LRRK2 expression in brain regions) and that could lead to a less accurate disease phenotype. It is noteworthy that non-symptomatic human carriers of the mutant LRRK2 gene have subtle abnormalities (elevated α-synuclein oligomer levels in cerebrospinal fluid, abnormal functional imaging in DAT-SPECT) but may not necessarily develop PD symptoms even in old age. Hence, our LRRK2 knockin mouse model can more accurately mimic human disease condition.

Mitochondrial dysfunction is a key feature in sporadic and familial PD. Accumulation of defective mitochondria and exposure to pesticides (eg rotenone) contribute to mitochondrial dysfunction. Mitochondria are key cellular organelles to supply energy by producing adenosine triphosphate (ATP). Nigrostriatal dopaminergic neurons are vulnerable to energy deficiency because of high energy-dependent activities (eg dopamine turnover) in their vast network of striatal presynaptic terminals. Hence, efficient mitochondrial ATP production in dopaminergic nerve terminals is crucial for survival. Pre-synaptic dysfunction in striatum is one of the earliest features of PD. We aim to develop a mutant LRRK2 mouse model of PD and investigate early disease processes (Fig. 1).

Results

To identify changes in mice with LRRK2^{R1441G} mutation

We have successfully generated LRRK2 knockin mouse colony, back-crossed to WT C57BL/6 mice for over eight generations to ensure similar genetic background as WT, apart from the mutation (Fig. 2a). Although most mutant LRRK2 human carriers are heterozygous, we used the homozygous knockin mice to accentuate the genetic risk. There were no obvious physical differences between mutant and WT mice even in old age. The mutant mice were fertile and had normal body weight and brain size. Histological examination did not show obvious dopaminergic neuronal loss or striatal dopamine transporter protein expression. There were no observable differences in the striatal synaptic structure between young WT and mutant mice under electron microscopy.

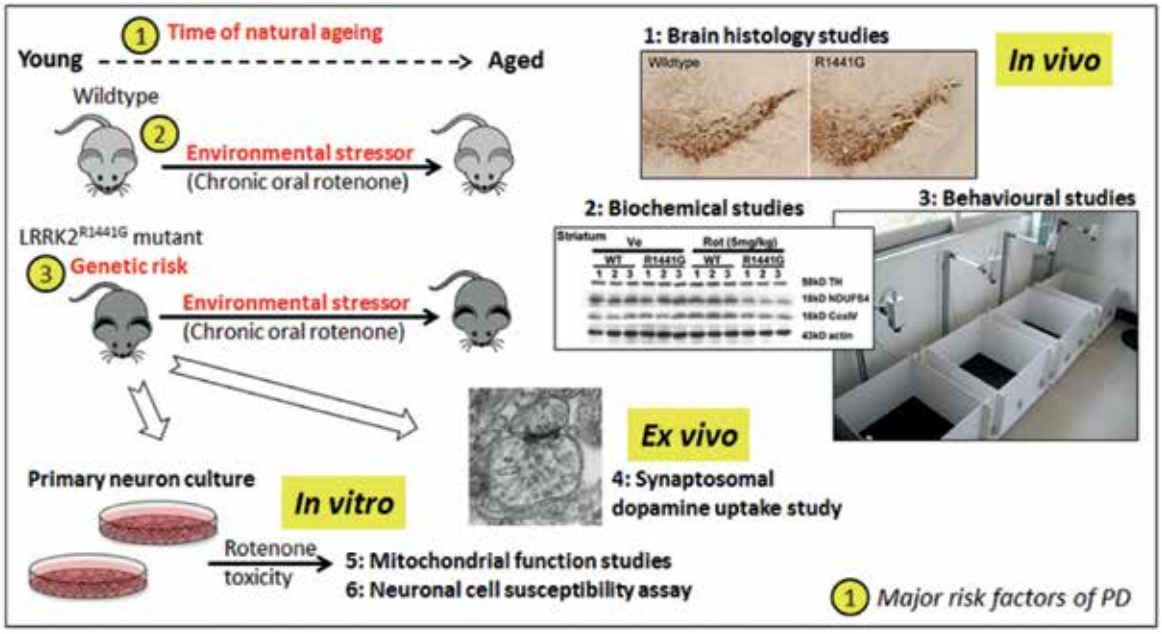

FIG 1. Overview of the current project scheme

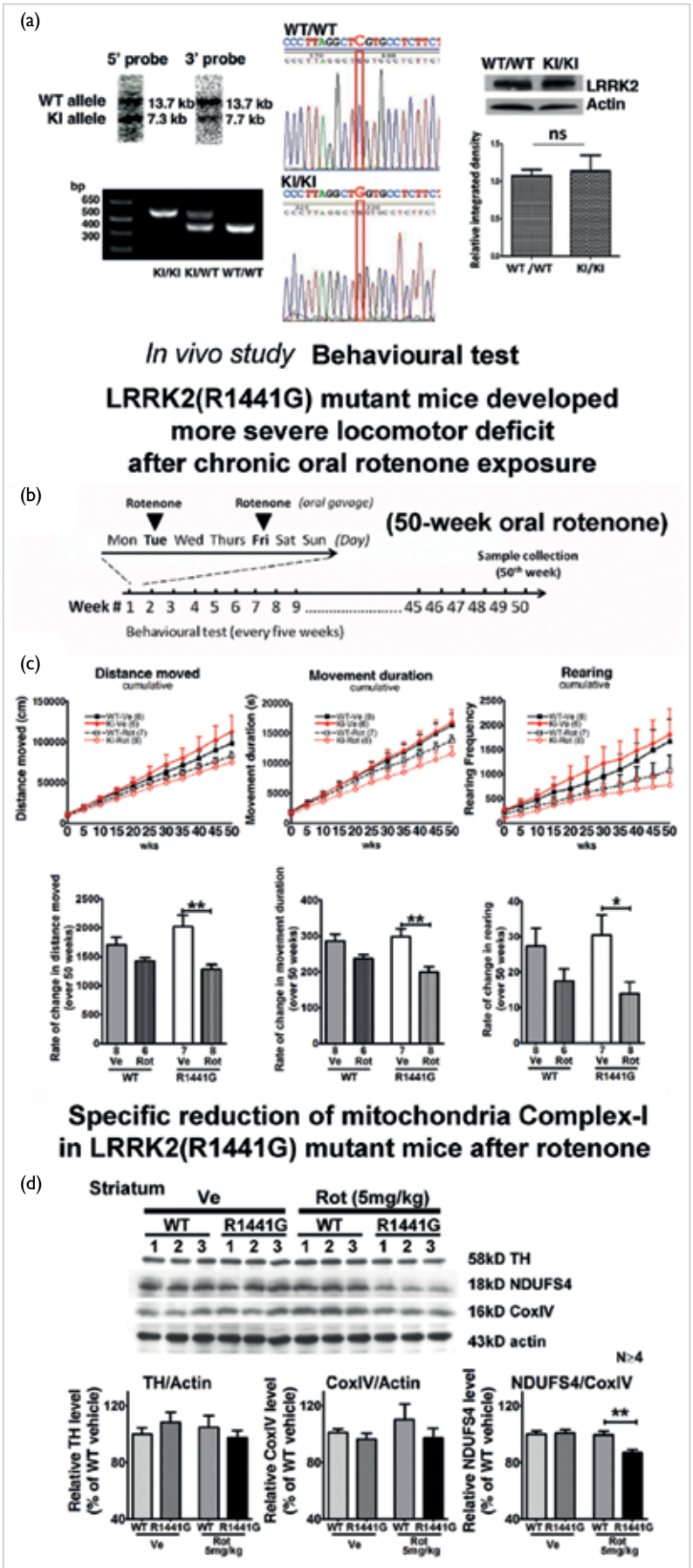

FIG 2. Successful generation of LRRK2^{R1441G} knockin mice and key findings of the Behavioural tests after chronic oral rotenone administration

To explore differences in neural signals in mutant mice

We explored striatal neurotransmission by measuring the local field potential using micro-electrode recording in vivo, stereotaxically positioned at dorsal striatum. Differences in amplitudes of the integrated neural signal between WT and mutant mice were assessed at three frequency bands: 3-8 Hz (theta), 13-30 Hz (beta), and 30-55 Hz (gamma). Our analyses showed no significant differences in the amplitudes of beta and gamma waves between young WT and mutant mice, although we observed a trend approaching significant difference for theta waves. These results need further evaluation on more mice, including aged mice. Although decreased amplitudes of theta waves are associated with anxiety, poor emotional awareness, and stress, the implications of these findings remain unclear. Interestingly, we observed that untreated mutant mice appeared more anxious than WT mice in the open-field behavioral test (by rearing frequency).

To determine whether the mutation confers genetic susceptibility against environmental stress

After we stressed the young mice using a single reserpine injection to cause temporary synaptic dysfunction, mutant mice had greater reduction of striatal synaptosomal dopamine uptake with more severe locomotor deficit (ie, less distance moved, velocity, and movement duration). Locomotor activity also recovered slower in mutant mice than in WT mice. These results indicate that LRRK2 mutation conferred a genetic susceptibility to striatal pre-synaptic dysfunction even at a young age.

We then explored whether mutant LRRK2 mice were susceptible to rotenone. Primary cortical and mesencephalic dopaminergic neuronal cultures from mutant mice were more susceptible to rotenone than WT mice, with more cell death and less energy supply (ATP) [Fig. 3a-e]. As striatal dopamine uptake into presynaptic nerve terminals is highly energy-dependent, the effects of rotenone on this process was investigated. Using striatal isolates containing presynaptic terminals, mutant mice had significantly lower dopamine uptake in striatal synaptosomes after rotenone toxicity (Fig. 3f-g), indicating that LRRK2 mutation conferred a genetic susceptibility to ATP depletion at the dopaminergic nerve terminals in striatum.

We then studied the effects of a combination of ageing, genetic susceptibility, and environmental toxicity. Small doses of oral rotenone were administered twice weekly over 50 weeks to reflect chronic environmental toxicity (Fig. 2b). Reduced Complex-I level was found in PD patients. We used rotenone because it is a specific mitochondrial

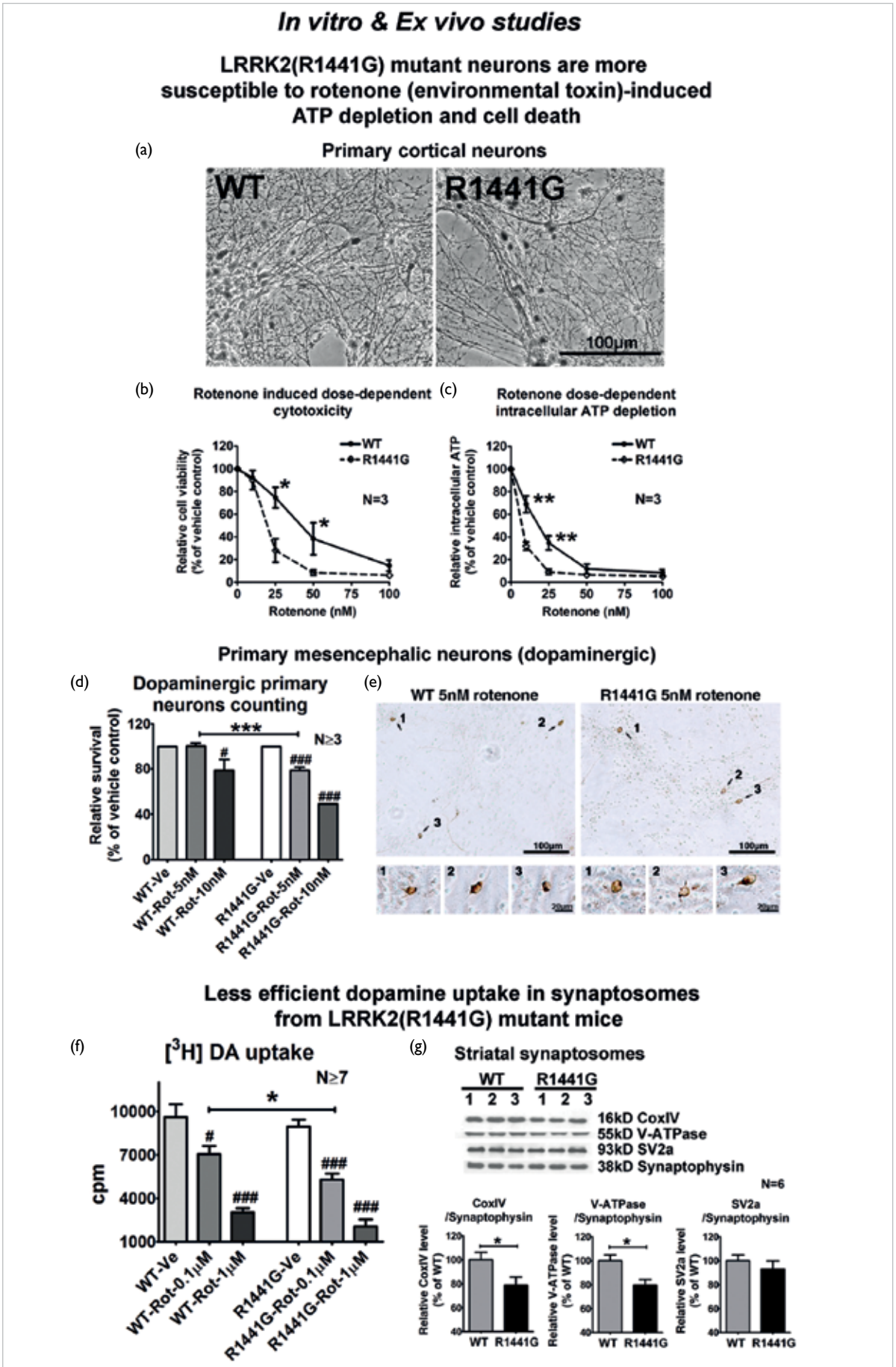

FIG 3. In vitro mitochondrial function studies and ex vivo dopamine uptake assay

Complex-I inhibitor that causes mitochondrial dysfunction. Ageing is inherent in this model because it was administered over half the normal lifespan of the mice.

Although the levels of apoptosis (programmed cell death) were similarly increased between mutant and WT mice after 50 weeks of oral rotenone, NDUFS4 (component of mitochondrial Complex-I) was significantly reduced in striatum of mutant mice, compared with WT mice, indicating that rotenone had a greater adverse impact on their mitochondria (Fig. 2d). We then assessed whether rotenone had any adverse effects on different aspects of locomotor activity of the mice by performing open-field tests every 5 weeks over 50 weeks. Interestingly, untreated mutant mice (ie, vehicle-treatment control) appeared more active than the WT mice, with greater cumulative distance moved and rearing frequency although the differences were not significant (Fig. 2c). Cumulative locomotor activity decreased in both WT and mutant mice treated with rotenone over 50 weeks. However, the decrease was significantly greater in mutant mice than WT mice. The cumulative distance moved, movement duration, and rearing frequency for rotenone-treated mutant mice over 50 weeks were significantly less than those for vehicle-treated mutant mice (Fig. 2c). However, rotenone-treated WT mice were not significantly affected by rotenone as compared with the vehicle-treated WT mice. These findings show that the LRRK2 mutation conferred a genetic susceptibility to rotenone toxicity.

To identify changes in gene expression in dopaminergic neurons

In collaboration with RIKEN, Japan, we cross-bred our mutant LRRK2 mice with their transgenic mouse that expresses tyrosine hydroxylase-gene-promoter specific GFP[2] to obtain a new colony of mice with LRRK2 knockin mutation and GFP fluorescent dopaminergic neurons. This allows direct visualisation of live dopaminergic neurons without immuno-labelling. Using fluorescence-activated cell sorting, we obtained pure dopaminergic neurons from aged mice and found that at least four genes were differentially expressed in our mutant mice compared with the age-matched WT mice. As these are highly purified dopaminergic neurons from substantia nigra, these differences would be of great interest to explore disease-causing pathways.

Significance and implications

The subtle abnormalities in the LRRK2^{R1441G} knockin mice are much more akin to human mutant LRRK2 carriers than existing transgenic or knockout models. Our model combines three vital risk factors to developing sporadic PD (ageing, genetic susceptibility, and chronic oral rotenone). We found susceptibilities and abnormal changes in our mutant LRRK2 mice. This enables testing of potential therapeutic agents on these mice to modify disease progression.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120976), the Henry G Leong Professorship in Neurology, and the Donation Fund for Neurology Research. The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Ho PW, Leung CT, Liu H, et al. Age-dependent accumulation of oligomeric SNCA/ α-synuclein from impaired degradation in mutant LRRK2 knockin mouse model of Parkinson disease: role for therapeutic activation of chaperone-mediated autophagy (CMA). Autophagy 2020;16:347-70.
2. Liu HF, Lu S, Ho PW, et al. LRRK2 R1441G mice are more liable to dopamine depletion and locomotor inactivity. Ann Clin Transl Neurol 2014;1:199-208.
3. Liu HF, Ho PW, Leung GC, et al. Combined LRRK2 mutation, aging and chronic low dose oral rotenone as a model of Parkinson's disease. Sci Rep 2017;7:40887.

References

1. Liu HF, Lu S, Ho PWL, et al. LRRK2 R1441G mice are more liable to dopamine depletion and locomotor inactivity. Ann Clin Trans Neurol 2014;1:199-208.
2. Matsushita N, Okada H, Yasoshima Y, Takahashi K, Kiuchi K, Kobayashi K. Dynamics of tyrosine hydroxylase promoter activity during midbrain dopaminergic neuron development. J Neurochem 2002;82:295-304.