Measuring LRRK2 kinase levels with Sandwich-ELISA in an *in vivo* viral model of LRRK2-associated Parkinson's disease

Ifigeneia Tsiakou, Alexia Victoria Polissidis
School of Liberal Arts and Sciences, The American College of Greece, 6 Gravias Street, 15342 Athens, Greece
Biomedical Research Foundation Academy of Athens, 4 Soranou tou Efesiou, 11527 Athens, Greece

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by motor control loss and movement impairments.PD can be attributed to both environmental and genetic factors. LRRK2 mutations, particularly G2019S-LRRK2 mutation, which increases LRRK2's kinase activity, contributes to most familial cases of PD. In this study, LRRK2 kinase activity is quantitatively investigated in an in vivo viral model of LRRK2 Parkinson's disease, overexpressing human wildtype or G2019S-LRRK2 in the striatum. Our findings aim to enhance our understanding of LRRK2 kinase activity in PD and explore its potential role as a biomarker.

INTRODUCTION

- Parkinson's disease (PD) is the most common movement disorder and the second most prevalent neurodegenerative condition, affecting over six million people worldwide.
- Key symptoms include bradykinesia, rigidity, and resting tremor, largely due to the loss of dopaminergic neurons in the substantia nigra and the formation of Lewy Bodies.
- A significant genetic factor in PD is the LRRK2 gene, particularly the G2019S-LRRK2 mutation, which increases the kinase activity of LRRK2 and is linked to both familial and sporadic cases.
- LRRK2 is a multifunctional protein involved in cellular processes such as vesicle trafficking and autophagy. LRRK2's enzymatic activities may be crucial for pathogenesis and LRRK2 kinase activity may be considered as a biomarker to track the development of PD.

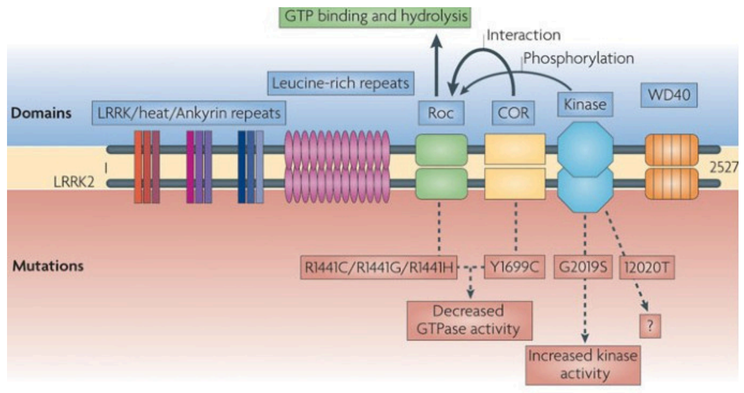


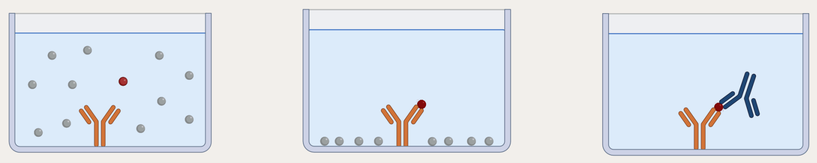
Figure 1. Structure of LRRK2 (adapted from Cookson M.R., 2010)

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MATERIALS AND METHODS

- Male wildtype (WT) mice (8-weeks old) were housed at BRFAA. They received unilateral, intrastriatal injections of HDhAd5 vectors (GFP, WT hLRRK2-FLAG, G2019S hLRRK2-FLAG) to promote neuron-specific expression in the nigrostriatal system.
- Three weeks post-injection, mice underwent behavioral evaluation and were sacrificed. Tissues from striatum were isolated, flash-frozen, and stored at –80°C.
- Striatal tissues were homogenized on ice with lysis buffer. Homogenates were centrifuged at 45,000 g for 1 hour at 4°C. Supernatants were stored at –80°C for ELISA.
- Sandwich ELISA was used to quantify LRRK2 levels. Plates were coated with capture antibody, washed, blocked with 1% BSA in TBST, and incubated with samples. Detection antibody was added, plates were washed, and substrate mixture was added. Plates were read using a luminescence reader.



RESULTS

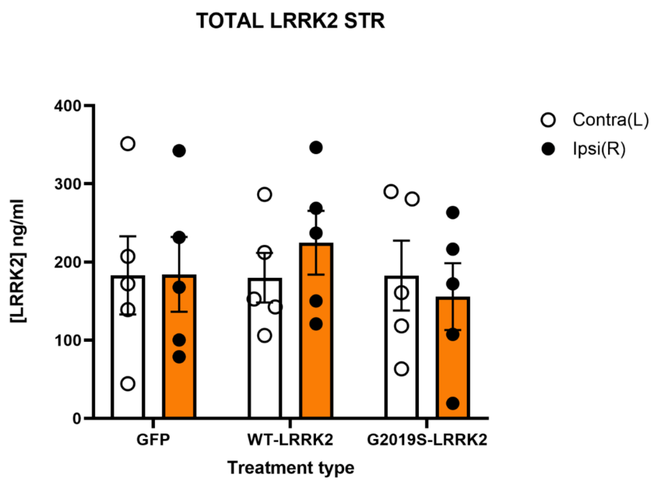


Figure 2. Total striatal LRRK2 levels

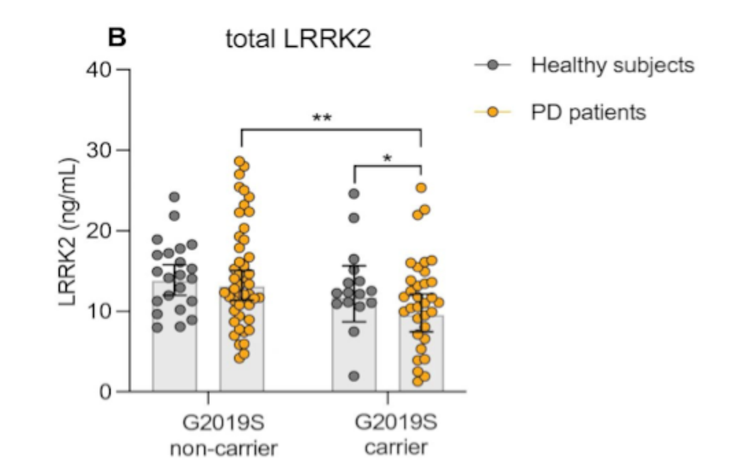


Figure 3. Total LRRK2 levels from PBMC samples in PD patients and healthy subjects by Wang et al. (2021)

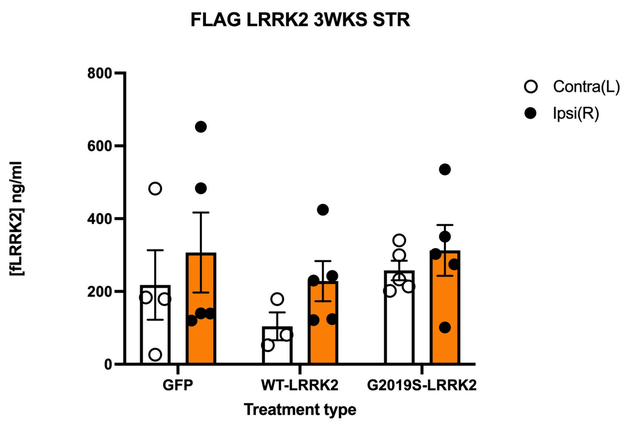
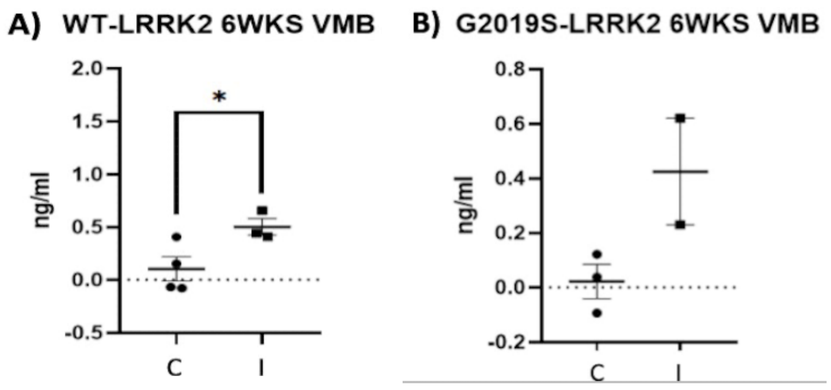


Figure 4. FLAG-tagged LRRK2 levels



Figures 5 and 6. Unpublished results of WT-LRRK2 and G2019S-LRRK2 levels at 6wks by Tantis-Tapeinos et al. (2022)

CONCLUSIONS

- Increased LRRK2 levels in WT mice striatum may indicate LRRK2's physiological importance in motor and dopamine control brain areas
- Wang et al. (2021) reported from PBMC samples that healthy G2019S non-carrier subjects had the highest LRRK2 levels between healthy carriers, PD non-carriers and PD carriers while lower LRRK2 levels were observed in PD patients carrying the G2019S mutation compared to healthy non- carriers
- G2019S-LRRK2 mutation may not have a major effect on LRRK2 levels in healthy subject but it is related with lower LRRK2 levels in PD pathophysiology
- In the analysis of FLAG-tagged LRRK2 levels the ipsilateral hemisphere, which was the injection site, exhibited higher levels in all treatments compared to contralateral hemisphere
- Tantis-Tapeinos et al. (2022) focused more on ventral midbrain region, but despite the regional disparity, the findings provide comparable insights into LRRK2 levels across brain regions involved in PD pathogenesis
- Comparison of 3wks and 6wks post injection LRRK2 levels is important to see what happens in earlier timepoints and assess for possibility as a biomarker
- Phospho-LRRK2 levels analysis is vital for PD pathology and future reassessment for accurate measurements for phospho-LRRK2 levels will be done
- The importance of LRRK2 kinase levels and its relationship to PD pathogenesis is crucial for understanding underlying mechanisms of the disease and developing target engagement biomarkers