Measuring *LRRK2* Levels in The Ventral Midbrain in a G2019S mutant *LRRK2*-associated Parkinson's Disease Mouse Model

Miranna Samlian[1], Ifigeneia Tsiakou[1], Hardy Rideout[2], Alexia Polissidis[1][2]

[1]Deree-The American College of Greece, [2]Biomedical Research Foundation Academy of Athens, Athens, Greece

Introduction

Parkinson's Disease (PD):

- A neurodegenerative disease characterized by loss of motor control and dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) of the brain's ventral midbrain (VMB) region.[1][2]
- Affects mainly individuals >60 years.[1]
- Majority of cases are idiopathic with familial cases making up 5-10%.[1]
- Early diagnosis is difficult as neurological symptoms (i.e.: memory issues, insomnia) can appear 20 years before motor symptoms and can be confused with other disorders, such as dementia.[1]
- Estimated that at least 6 million people suffer from PD globally.[1]
- No cure – treatment mainly symptomatic.[1][2]
- No biomolecular diagnostic test available.[1][2]

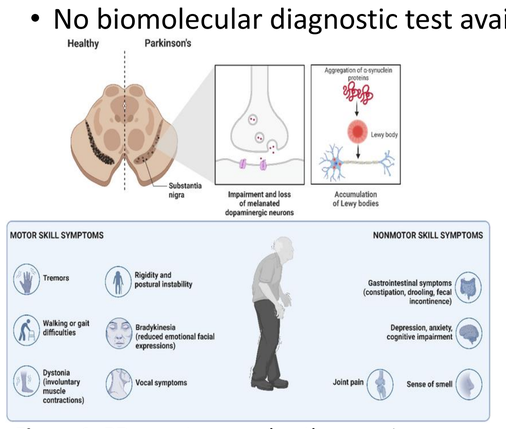


Figure 1. PD symptoms and pathogenesis.
Image Credit: Dias-Carvalho et al. (2023).

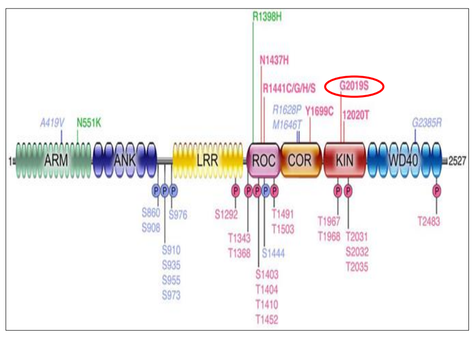


Figure 2. Functional domains of *LRRK2* protein. In red, the region studied – G2019S.
Image Credit: Rideout et al. (2020)[2]

Leucine Rich Repeat Kinase 2 *(LRRK2):*

- Large gene (280 kDa) and most common genetic cause of PD.[1][2][3]
- G2019S is the most common pathogenic mutation.[1][2]
- Irregular activity present in familial PD and idiopathic PD.[2][3]
- Codes for kinase protein and is present in multiple parts of the body (i.e.: brain, liver, immune cells).[1][2][3]
- Has many functions due to kinase activity, including autophagy and signal transduction.[1][2][3]
- Mutations in gene cause increased autophosphorylation of protein and its substrates → increased kinase function.[1][2]
- Studied as potential biomarker and drug target due to kinase function and role in vesicular trafficking.[1][2][3]

Previous Studies:

- Development of helper-associated adenoviral (hAd5) G2019S-*LRRK2* mouse model.[4][5]
  - Stereotaxic injection of adenovirus to induce expression of *LRRK2* in mouse brain (striatum and ventral midbrain)
  - Superior to other mouse models
  - Time-saving (vs aged transgenic mouse models)
  - Constructive validity (good model of PD pathogenesis)
- Two studies:
  - Nguyen et al. (2020)[4]
    → Assessed *LRRK2* levels 3 weeks post-injection in the striatum
    → Stereology & Western Immunoblotting
  - Previous, unpublished study on mouse model developed at BRFAA.[5]
    → Assessed *LRRK2* levels 6 weeks post-injection
    → Stereology, Western Immunoblotting, & ELISA

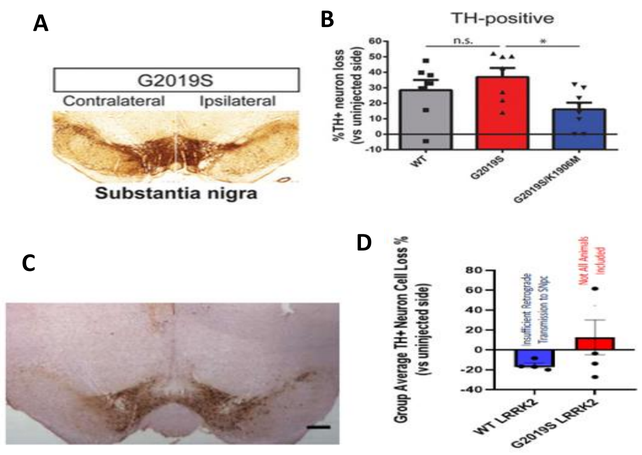
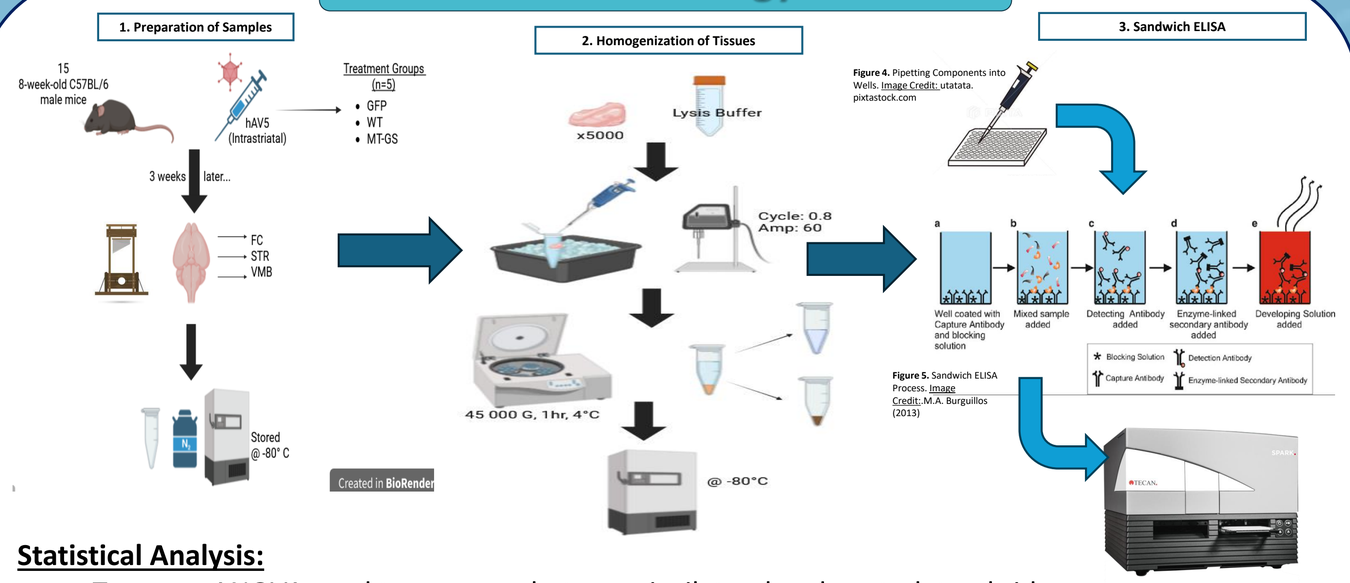


Figure 3. Immunostaining of SNpc (A,C) and measure of TH+ neuronal loss (B,D). (Above row) Nguyen et al. (2020) with measurements performed 3 weeks post-injection in rats (A,B). (Bottom row) The unpublished study using the hAV5 G2019S-associated mouse model with measurements performed 6-weeks post-injection (C,D). In both cases, higher dopaminergic neuronal loss is observed in the SNpc with higher levels of loss associated with the mutant G2019S-LRRK2 groups.

Objective

- To further characterize the hAd5-associated *LRRK2* PD mouse model by measuring *LRRK2* levels and phosphorylation 3-weeks post-injection.
- In-house Sandwich ELISA analysis used to measure total *LRRK2,* exogenous *LRRK2* (flag-tagged), and phosphorylated at the S935 region *LRRK2* (p*LRRK2)* levels in ventral midbrain (VMB) of mice.
- In-house established ELISA was used as it is more sensitive and has higher throughput than Western Immunoblotting.

Methodology

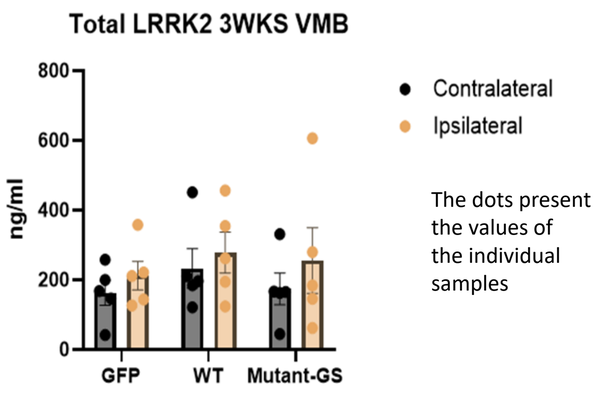


Statistical Analysis:

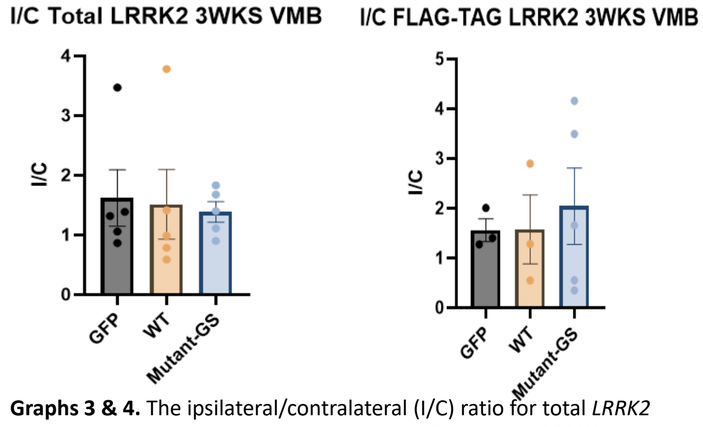
- Two-way ANOVA used to compare between ipsilateral and contralateral sides
- One-way ANOVA was used to compare between the three treatment groups.

Results

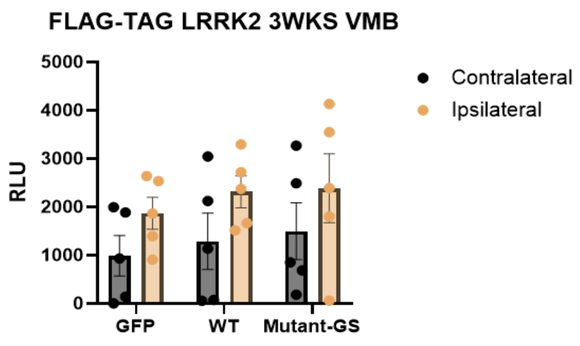
- Slight increases in *LRRK2* levels were observed on the ipsilateral side (where the hAd5 was injected) between the three treatment groups in both the total *LRRK2* and flag-tagged measurements (Graphs 1,2). The ratio (I/C) was calculated to get the overall amount of *LRRK2* in the VMB.
- Phosphorylated LRRK2 measurements had a high background signal (likely due to their expiration) and were thus not further analysed) to be abnormal.



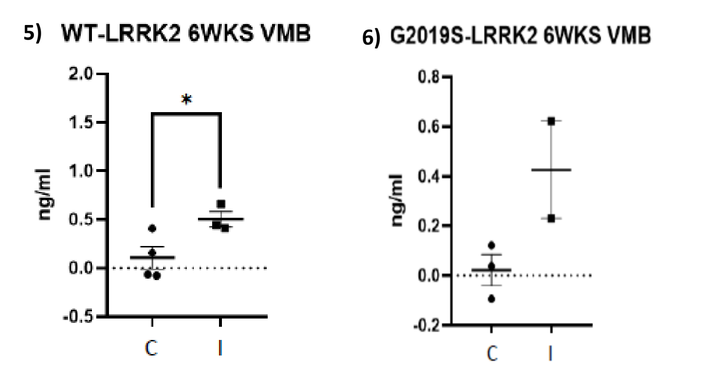
Graph 1. Total LRRK2 concentrations in the VMB three weeks post-injection. Although slight increases in *LRRK2* concentrations were observed on the ipsilateral side in all the treatment groups, those differences were found to be insignificant (p = 0.1459, DF = 2.594).



Graphs 3 & 4. The ipsilateral/contralateral (I/C) ratio for total *LRRK2* levels between treatment groups (left), and flag-tagged *LRRK2* levels between treatment groups (p=0.8519, DF=0.1635) (right). As with the previous measurements, increases were observed, but found statistically insignificant (For I/C total *LRRK2:* p = 0.9478, DF=0.054; For I/C flag-tagged *LRRK2*: p = 0.8519, DF=0.1635)



Graph 2. Exogenous flag-tag LRRK2 levels in mouse VMB 3 weeks post-injection. As with graph 1, increases were observed on the ipsilateral side in all three treatment groups, however from statistical analysis these differences were found to be insignificant (p = 0.0327, DF = 6.647; *ad hoc* analysis found that there was no significant difference between the hemispheres).



Graphs 5 & 6. Graphs taken from unpublished study measuring *LRRK2* levels 6-weeks post-injection. They were compared to Graph 2 (far left)). In comparison to Graph 2, the previous results demonstrate a significant increase in flag-tagged LRRK2 levels in wild-type LRRK2-injected mice(5) and a non-significant increase in mutant G2019S-LRRK2 mice VMBs 6-weeks post-injection (6).

Discussion & Conclusion

- Due to conjugate not being fresh, p*LRRK2* ELISA readings were disregarded from further analysis; a rerun of this measurement with a fresh conjugate will be performed.
- In all measurements, slight increases of *LRRK2* expression were observed, however the differences were found to be insignificant.
- In comparison to the previous study where *LRRK2* levels were measured 6-weeks post-injection, the results were found to be more robust, indicating that there is greater viral transduction after 6-weeks rather than 3-weeks.
- The differences in this current study may be insignificant due to anatomy of VMB and how it is difficult to isolate the SNpc, which may cause a 'dilution' effect and the potential loss of dopaminergic neurons, potentially affecting *LRRK2* activity.
- To conclude, there is insufficient viral transduction at this time point, however this early timepoint may have use in determining neurodegenerative pathways and possible preventative treatments.
- Further research is needed, including re-assessing p*LRRK2* levels, dopamine neuron stereology, and increasing cohort size.

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