Dysregulation of PLA2G6 activity induces degeneration in astrocytes in culture

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Introduction

PLA2G6 activity dysfunction causes neuroaxonal degeneration with iron accumulation, which during childhood is lethal, and in adulthood may induce Parkinsonian-like disease (1). Recently, it was shown that fibroblasts from idiopathic or sporadic Parkinson's disease patients show impaired store-operated calcium entry (SOCE) (2). SOCE controls the activity of Calmodulin, a putative negative regulator of PLA2G6, so the impairment of intracellular calcium flux suggests a role for PLA2G6 in the PD pathology. Here we test the hypothesis that unbalanced activity of PLA2G6 in astrocytes induces deleterious effects on human dopaminergic cells because of their failure to respond with pro-survival/anti-inflammatory signaling to prevent dopaminergic neuronal death and microglia activation.

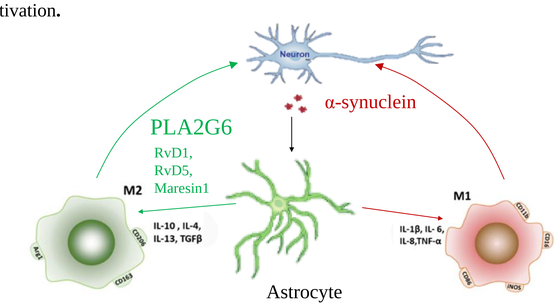

Figure 1. α-synuclein from the dopaminergic neurons activate a toxic response by the astrocytes. Because PLA2G6 hydrolyzes DHA from phospholipids to be converted into RvD1, RvD5, and Maresin1, when it is dysfunctional these bioactive lipid mediators are lacking and the inflammation is not resolved. The microglia receive pro-inflammatory signals causing the M1 phenotype characterized by the release of toxic cytokines such as TNF- α.

Methods

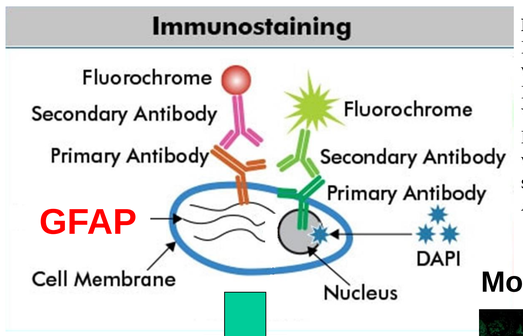

Figure 2: Methods used for the project: Immunostaining: to target astrocytes we use GFAP primary antibody. Images were taken using Olympus Fluo View 1200 confocal microscope. Morphological analysis was performed with Imaris 9.6 (Bitplane, UK) and statistical analysis using one way Anova and T-test

Results

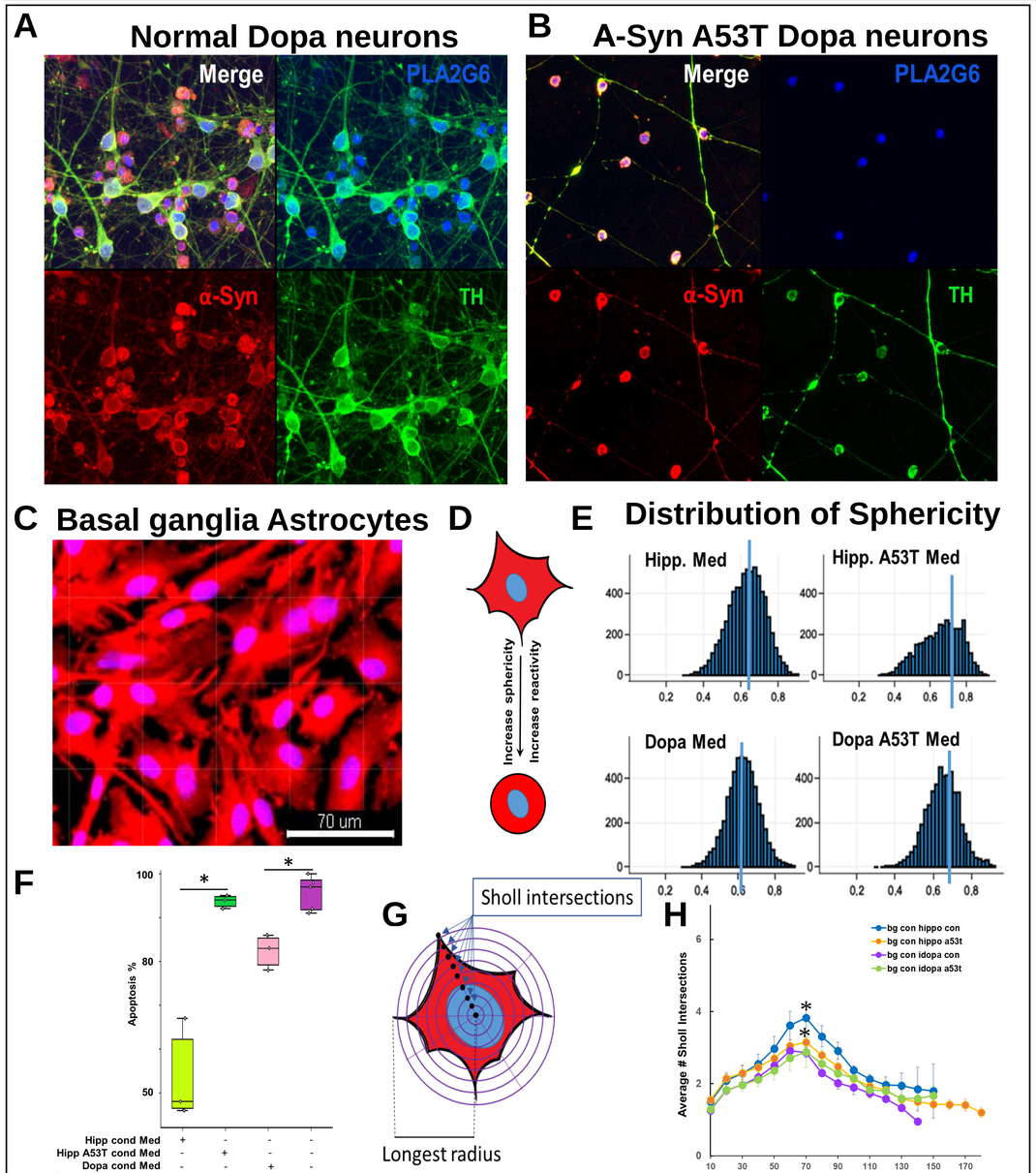

Figure 3 Astrocytes are activated by dopaminergic neuron conditioned medium. **A**. Normal human dopaminergic neurons **B**. Human dopaminergic neurons expressing mutated α-synuclein **C**. Normal rat astrocytes **D**. Representation of transition from normal to spherical phenotype **E**. Change in sphericity due to exposure of astrocytes to neuronal conditioned media **F**. Apoptosis of astrocytes exposed to various conditioned media **G**. Schematization of Sholl intersections technique **H**. Sholl analysis plots for astrocytes exposed to conditioned media. PLA2G6 stained blue, α-synuclein stained red, and Tyrosine Hydroxylase stained in green *p<0.05

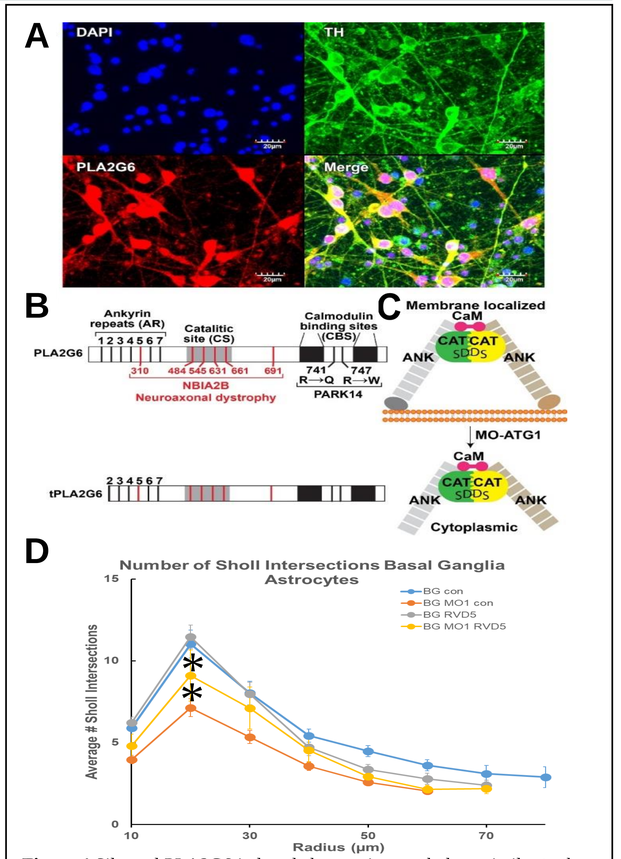

Figure 4 Silenced PLA2G6 induced changes in morphology similar to those observed in the activation of astrocytes **A**. Normal human dopaminergic neurons express PLA2G6. **B**. PLA2G6 mutations **C**. change in structure from normal to silenced PLA2G6 **D**. Sholl analysis of astrocytes in which PLA2G6 was silenced and rescued with the downstream bioactive lipid mediator RvD5. *p<0.05

Conclusions

- PLA2G6 dysfunction affects the production of lipid mediators such as RvD1, RvD5, and Maresin 1
- A53T mutated α-synuclein causes activation of astrocytes to the pro-inflammatory pathway
- PLA2G6 induces changes in morphology of astrocytes resembling the activation into pro-inflammatory phenotype

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