MOL231: Comparative analysis of WT and DJ-1 knockout lipid profiles using the Zebrafish model for Parkinson's Disease

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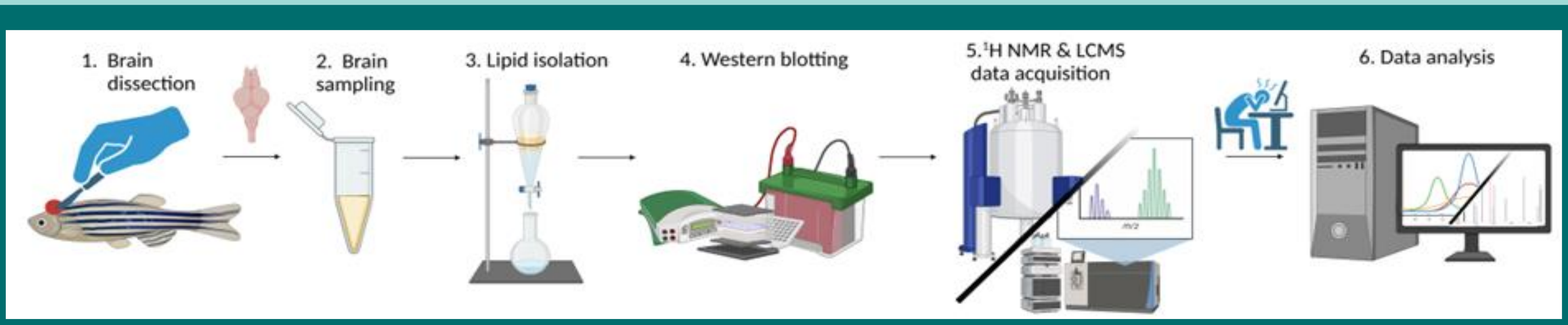
Introduction: Parkinson's Disease is the second most common neurodegenerative disease worldwide, with progressive cell loss confined mostly to dopaminergic neurons of the substantia nigra (Anglade et al., 1997). One way of modelling Parkinson's disease in research is to deprive tissue of DJ-1, a protein encoded by the PARK7 gene. Although the key molecular events leading to Parkinson's Disease remain unknown, protein misfolding and oxidative stress are implicated. DJ-1 has several functions that play an important role in cell protection against oxidative stress and cell death acting as an oxidative stress sensor and redox-sensitive chaperone and protease (Bretaud et al., 2007). It is also reported to influence metabolism, including lipid metabolism (Mencke et al., 2021). Here, we study the lipid profiles of wildtype (WT) and knock-out (KO) in zebrafish (*Danio Rerio*) where we created a comparative analysis. The study used lipid extraction, SDS-PAGE gel-electrophoresis, Western blotting, ^{1}H NMR, and Ultra Performance Liquid Chromatography – High Resolution Mass Spectrometry (UPLC-HRMS/MS, LCMS).

Methods



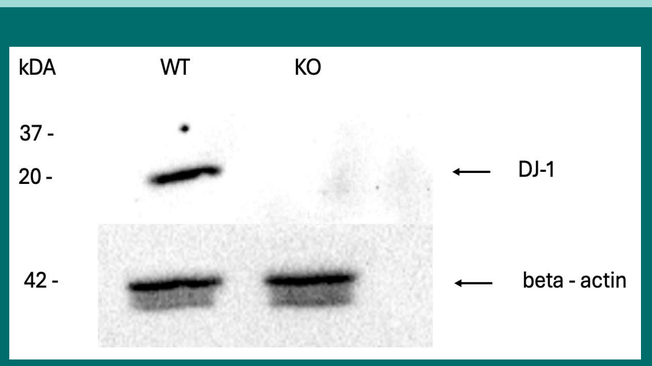


Fig. 1: Western blot of WT and DJ-1-KO with 1:3000 anti-DJ1 rabbit primary antibody. 20 µg WT and 20 µg KO were loaded. An anti-beta-actin mouse was used as a negative control. The control had strong bands for both conditions, indicating that the DJ-1 protein is absent in the KO.

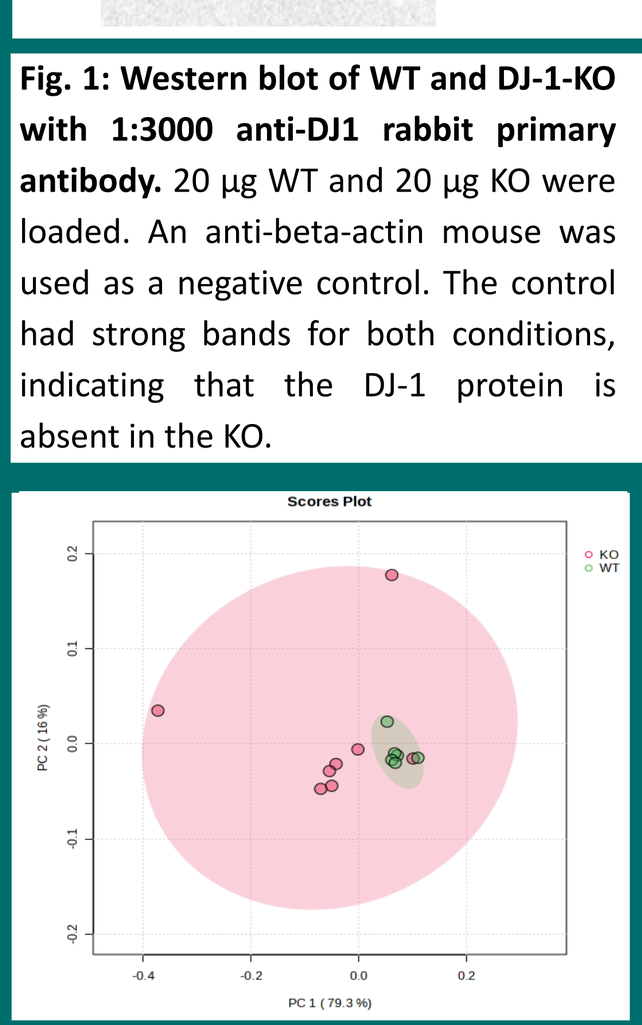
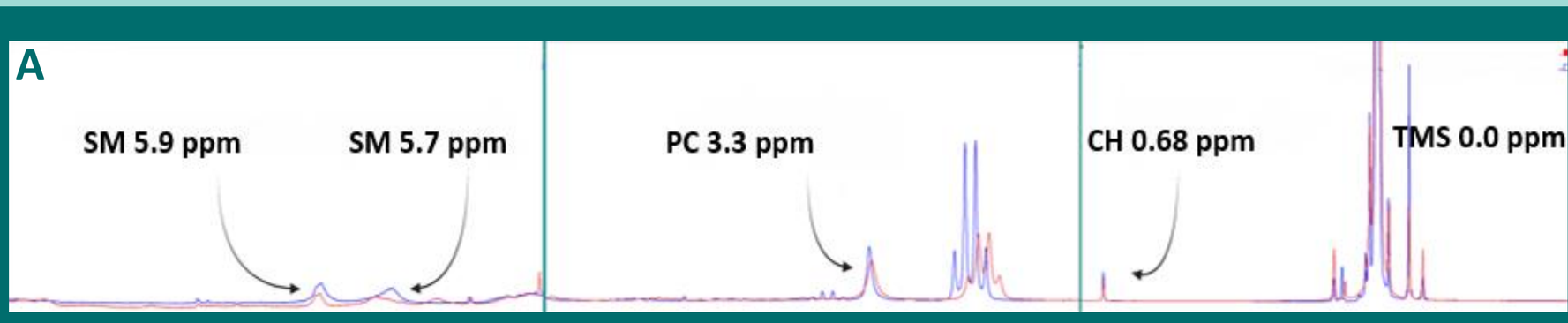
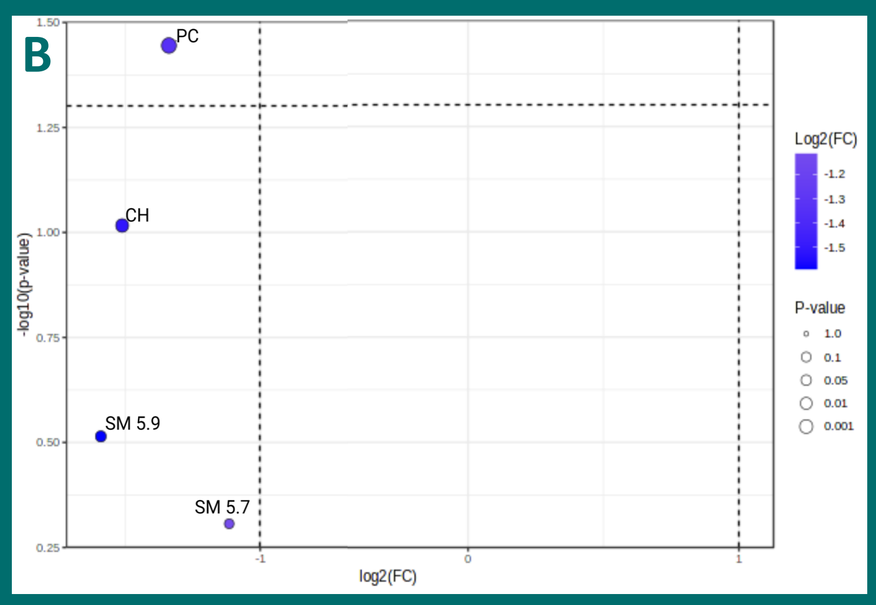


Fig. 3: PCA Score plot WT and DJ-1-KO.

Fig. 4: Comparison of representative lipids in WT and DJ-1-KO. (A) PC and (B) CH in ^{1}H NMR, and (C) PC 18:2, (D) PE 18:1, (E) TG 16:1 in LCMS from the volcano plots.





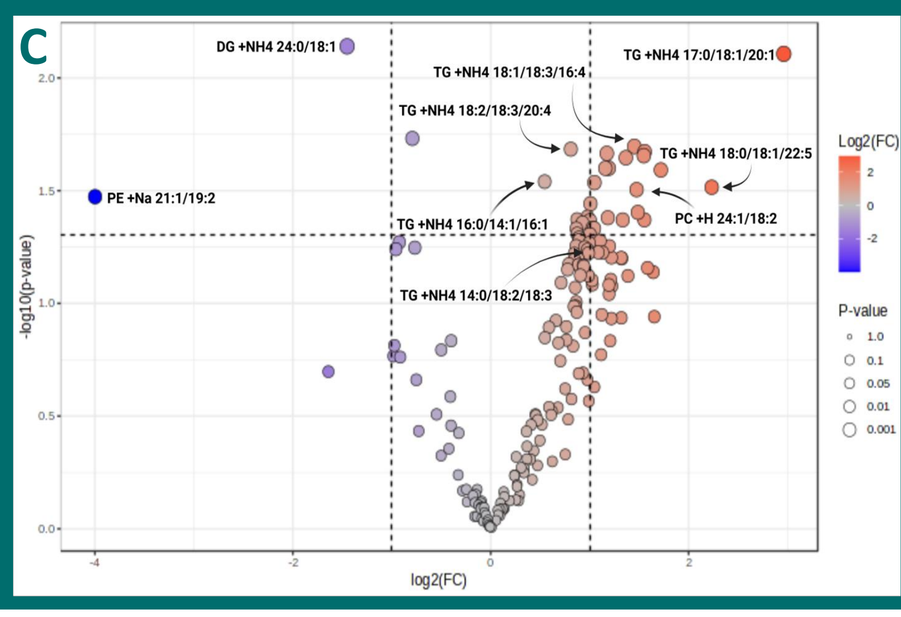
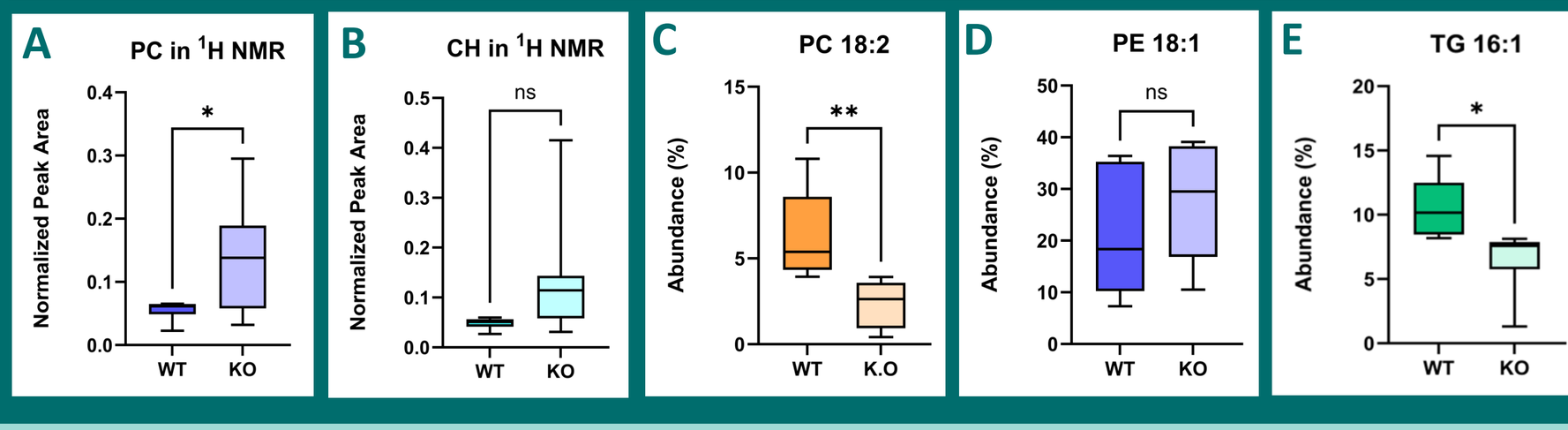


Fig. 2: (A) Overlay WT and DJ-1-KO ^{1}H NMR spectra. Peaks (ppm) of the lipids of interest: Cholesterol 0.68 (CH), Phosphatidylcholine 3.3 (PC), and Sphingomyelin 5.7 and 5.9 (SM) with trimethylsilyl (TMS) as internal standard. **(B) and (C) Volcano plot with lipids of interest.** The Y-axis plots the (B) normalized peak area [abs*Hz] for each lipid, and (C) means of m/z with their means of retention time. When pairing these different means together, nine lipid species were found either significantly upregulated (orange) or downregulated (blue) in KO compared to WT.



References: Anglade, P., Vyas, S., Javoy-Agid, F., Herrero, M. T., Michel, P. P., Marquez, J., Mouatt-Prigent, A., Ruberg, M., Hirsch, E. C., & Agid, Y. (1997). Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histology and histopathology*, *12*(1), 25–31.

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Conclusion:

- **PC is significant in NMR according to the volcano plot.**
- **PC 18:2 is significant in LCMS. This fatty acid chain can be seen in one of the upregulated lipid species in the volcano plot, PC +H 24:1/18:2.**
- **TG is significantly downregulated, underscoring the Parkinson's Disease link with lipid metabolism.**
- **Cholesterol (not significant) has been noted as relevant as a possible risk factor in Parkinson's Disease, but this question remains open.**