

## INTRODUCTION

- Alteration in the expression of microtubular proteins associated with microtubule dynamics and neuronal plasticity has been linked with the pathogenesis and treatment of major depressive disorder (MDD) [1].
- One third of MDD patients are unresponsive to antidepressant drugs, a recognised subtype of MDD known as treatment resistant depression (TRD).
- The endogenous 'depressed' Wistar Kyoto (WKY) rat is unresponsive to Selective Serotonin Reuptake Inhibitors (SSRIs) and was used here as a purported model of TRD [2].
- Acetylated  $\alpha$ -Tubulin (Acet-Tub) is associated with less dynamic microtubules and was found to be increased in the hippocampus in a rat model of depression [3].
- Likewise, a higher ratio of tyrosinated  $\alpha$ -Tubulin (Tyr-Tub) to detyrosinated  $\alpha$ -Tubulin (Glu-Tub) is a marker of more dynamic microtubules and has been found to be decreased in models of depression [3].
- To date no biomarker of disease or pharmacological efficacy has been identified in TRD.
- Aim: To explore the feasibility of plasma Acet-Tub and Tyr/Glu-Tub expression as biomarker in TRD, using plasma samples from a depressed and non-depressed cohort.**

## METHODS

**Human Plasma:** Human plasma was obtained as part of an ongoing study into TRD conducted by Dr. Declan McLoughlin's team. Equal number of male and female participants were used for each group. Samples were prepared with a protein concentration of 1  $\mu\text{g}/\mu\text{l}$ .

**Rat Plasma:** WKY rats (TRD model) and SD rats ('healthy' control) were sacrificed by decapitation and trunk blood was collected. Plasma was isolated from blood samples by centrifugation. Plasma samples were preserved using a protease inhibitor cocktail and stored at  $-80^\circ\text{C}$ . Samples were prepared with a protein concentration of 1  $\mu\text{g}/\mu\text{l}$ .

**Infrared Western Blotting (IFWB):** The expression of plasma Acet-Tub, Tyr-Tub, and Glu-Tub was measured using a protocol of IFWB adapted from previous studies [3]. Acet-Tub, Tyr-Tub, and Glu-tub were analysed using an Odyssey Infrared imaging system. Proteins were transferred onto PVDF membranes after electrophoresis and blocked with Odyssey Blocking Buffer.

## CONCLUSION

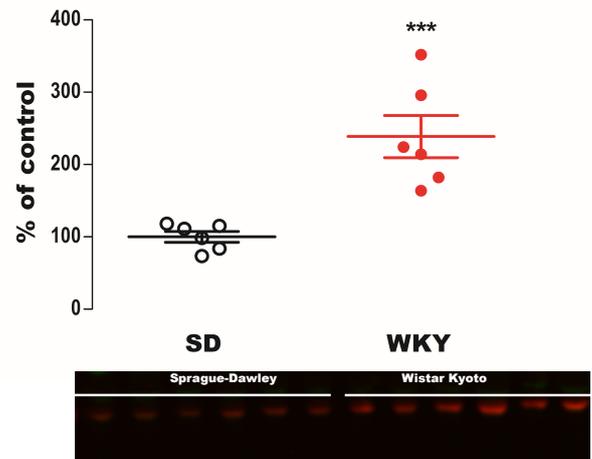
- Previous work shows the WKY rat to be a suitable model for TRD [1].
- WKY rats exhibit consistent overexpression of plasma Acet-Tub in line with the previous observed decreased hippocampal microtubular dynamics [4].
- Despite low sample size, greater variability and relative increase in Acet-Tub expression can be seen in depressed individuals, suggesting less stable microtubules
- Likewise, the lower Tyr/Glu ratio in depressed individuals is indicative of less dynamic microtubules.
- These findings in human plasma are analogous to the WKY rat where plasma and brain microtubular alterations were recorded [4].
- Larger samples sizes are often need in human studies to accommodate greater variability in protein expression. Work is underway to further validate our findings.
- Plasma Acet-Tub Expression and Tyr/Glu ratio may represent potential biomarkers of disease progression and treatment responsiveness in TRD.**

## REFERENCES

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## RESULTS

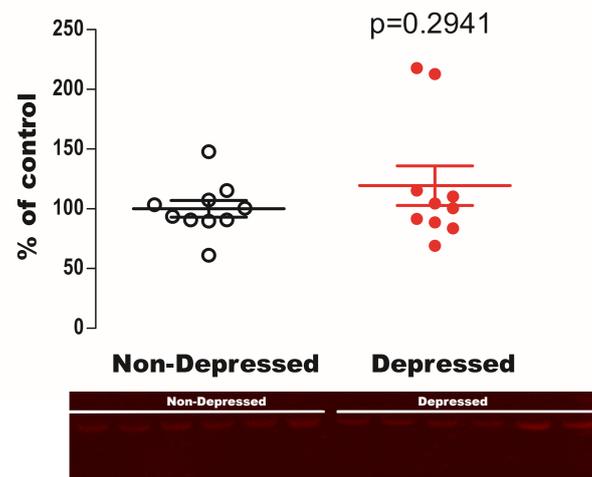
(1)



**Figure 1. Plasma Acet-Tub expression in Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats**

WKY rats (n=6) overexpressed plasma Acet-Tub compared with SD (n=6) (\*\*\*)  $p < 0.001$ . Student's t-test. Data: Mean  $\pm$  SEM.

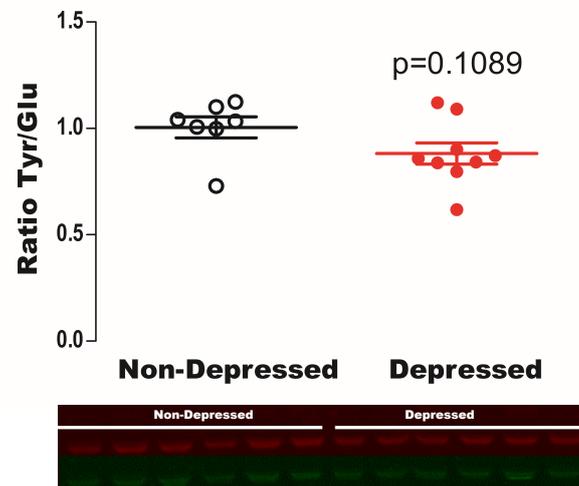
(2)



**Figure 2. Plasma Acet-Tub Expression in Depressed and non-Depressed Individuals**

Plasma taken from depressed individuals (n=10) shows a relatively higher expression of Acet-Tub and greater expression variability compared to non-depressed individuals (n=10) ( $p=0.2941$ ). Student's t-test. Data: Mean  $\pm$  SEM.

(3)



**Figure 3. Plasma Tyr/Glu Expression in Depressed and non-Depressed Individuals**

Plasma taken from depressed individuals (n=9) shows a relatively lower Tyr-Glu ratio and greater variability compared to non-depressed individuals (n=7) ( $p=0.1089$ ). Glu-Tub red, Tyr-Tub green. Student's t-test. Data: Mean  $\pm$  SEM.