© 2023. Published by The Company of Biologists Ltd | Disease Models & Mechanisms (2023) 16, dmm050220. doi:10.1242/dmm.050220

FIRST PERSON

# First person – Rebeka Popovic and Yizhou Yu

First Person is a series of interviews with the first authors of a selection of papers published in Disease Models & Mechanisms, helping researchers promote themselves alongside their papers. Rebeka Popovic and Yizhou Yu are co-first authors on 'Upregulation of Tribbles decreases body weight and increases sleep duration', published in DMM. Rebeka conducted the research described in this article while a PhD student in Dr Luis Miguel Martins' lab at the University of Cambridge, Cambridge, UK. Her PhD investigated mechanisms of neurodegeneration, with a focus on endoplasmic reticulum stress and mitochondrial dysfunction, as well as inter-organ communication in disease. She is now a postdoc in the lab of Prof. Fiona Ducotterd at the Drug Discovery Institute at University College London, London, UK, exploring new therapeutic targets for the treatment of neurodegenerative diseases. Yizhou is a PhD student in the lab of Dr Luis Miguel Martins at the University of Cambridge, Cambridge, UK. His research involves combining computational biology and wet-lab techniques to understand the genetic and environmental causes of age-related diseases.

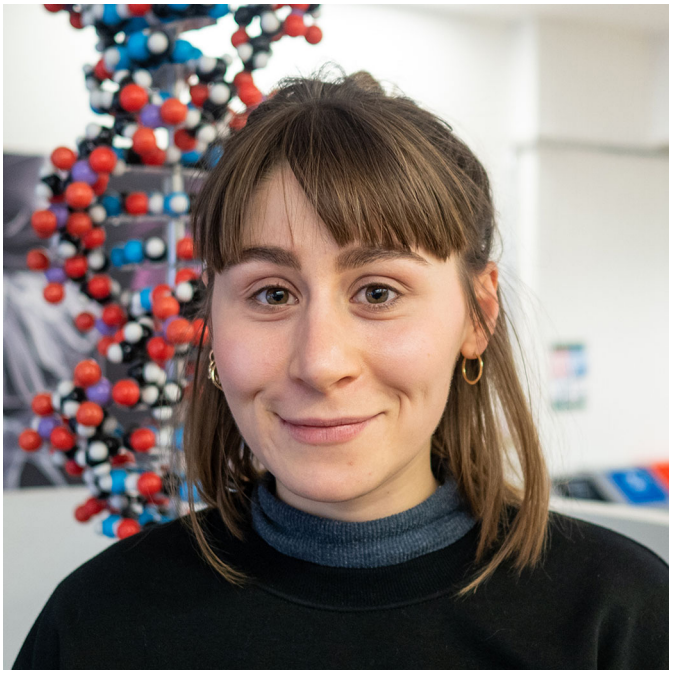
**How would you explain the main findings of your paper to non-scientific family and friends?**

**RP:** Obesity represents a major current global health problem. It is characterized by increased accumulation of lipids in adipose tissue and insulin resistance (when cells don't respond properly to insulin), which can lead to the development of type 2 diabetes mellitus (T2DM). This is a concern as T2DM leads to metabolic abnormalities, poor sleep and an increased risk of mortality in adults. Previous studies have shown that alterations in insulin receptor signalling (IRS), a pathway which controls how the nutritional signals are translated to body metabolism, lead to insulin resistance and T2DM. The Tribbles pseudokinases, a family of adaptor proteins that modulate the activity of many regulatory proteins, have been demonstrated as important regulators of the IRS pathway, and a mutation in one of the human tribbles pseudokinase genes has been reported as a risk factor for T2DM. Given the role of Tribbles in the regulation of pathways linked to T2DM, we asked if manipulating its levels can impact insulin signalling, body weight, sleep and lifespan. First, using the fruit fly, we show that expressing Tribbles in the fat body, the fly organ analogous to the human liver and adipose tissue, results in decreases in fly body weight, lifespan and systemic insulin levels, and an increase in sleep duration.

**"[…] expressing Tribbles in the fat body […] results in decreases in fly body weight, lifespan and systemic insulin levels, and an increase in sleep duration."**

Rebeka Popovic's contact details: Alzheimer's Research UK UCL Drug Discovery Institute, University College London, The Cruciform Building, Gower Street, London, WC1E 6BT, UK.
Yizhou Yu's contact details: MRC Toxicology Unit, University of Cambridge, Gleeson Building, Tennis Court Road, Cambridge CB2 1QR, UK.
E-mail: rebeka.popovic@ucl.ac.uk; yizhou0421@gmail.com





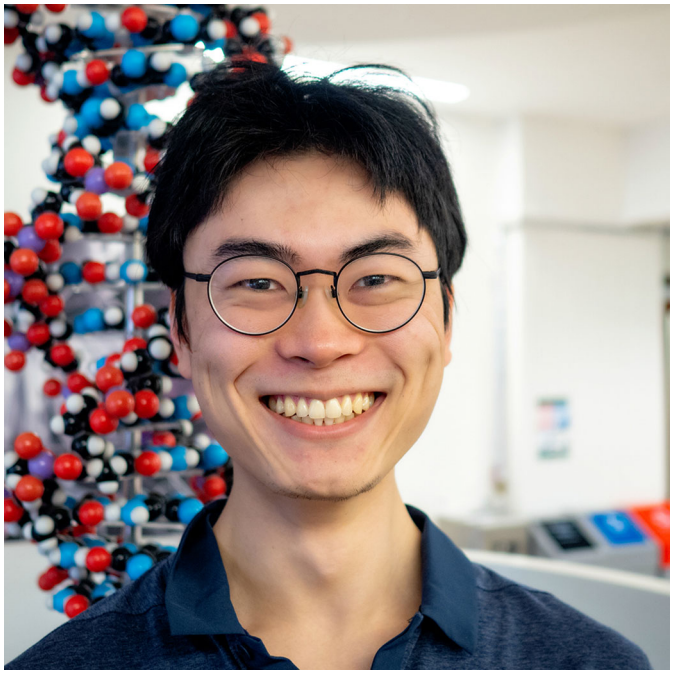
Rebeka Popovic

**YY:** Sleep and obesity are major factors that affect our quality of life. Based on the exciting results from fruit flies, we then wondered whether the human versions of Tribbles could also be linked to sleep and weight. We hypothesised that genetic variations in Tribbles were linked to body weight and sleep patterns in humans. Genetic variations occur naturally. We identified some that are associated with the expression of Tribbles and discovered that when a genetic variant was linked to higher mRNA levels of Tribbles, it was also associated with longer sleep duration and lower body weight. The reverse was equally true. We thus concluded that Tribbles may play a role in regulating body weight and sleep patterns in humans. Additionally, we identified a single variation in a Tribbles gene that was particularly interesting. This variation (rs2295490) is a well-known variant that's linked to insulin, blood pressure and diabetes in human patients. We honed in on this genetic variation and saw that it corroborated our analysis on sleep and obesity in humans. We also introduced this mutation in flies and observed that flies with this mutated version of Tribbles have a similar sleep pattern to humans.

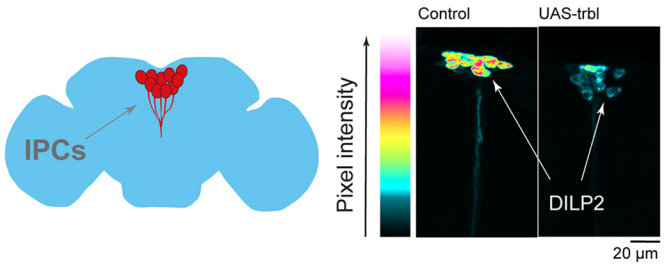
**What are the potential implications of these results for your field of research?**

**RP:** Previous studies have shown that *Drosophila* Tribbles acts like a negative regulator of insulin signalling, by inhibiting the Akt kinase, a downstream target of insulin. Overexpression of Tribbles in the fat body mimicked the metabolic defects induced by a high-fat diet, and its downregulation alleviated the metabolic defects associated with this diet, indicating that Tribbles is a key mediator of insulin resistance. We have expanded on these results, showing that expression of Tribbles in this fly organ further

Disease Models & Mechanisms (2023) 16, dmm050220. doi:10.1242/dmm.050220



Yizhou Yu




**Tribbles expression reduces *Drosophila* insulin-like peptide 2 (DILP2) levels.** Schematic representation of the anatomical location of insulin-producing cells (IPCs; red) in the adult fly brain (left). A reduced level of DILP2 was detected in IPCs of flies expressing Tribbles in the fat body (intensity levels shown as a five-tone heat map) (right).

modulates lifespan, body weight and sleep, important markers of T2DM. Through inter-organ communication, the fat body can control the release of insulin from the insulin-producing cells (IPCs) in the brain via humoral signalling, and our study suggests Tribbles as another molecule which may regulate this interorgan communication.

**YY:** In humans, we confirmed that genetic variations in the Tribbles genes are associated with T2DM. Weight changes and sleep disturbances are common features of many diseases, including Alzheimer's disease. Ageing also causes changes in weight and sleep patterns. Since we identified Tribbles as a key mediator of sleep and obesity, it is possible that genetic differences in the expression level of Tribbles could affect age-related diseases. Similarly, the activity or expression of Tribbles could change with age and cause disturbances in sleep and weight. Future studies could investigate the effects of Tribbles in aged and diseased animals.

### What are the main advantages and drawbacks of the experimental system you have used as it relates to the disease you are investigating?

**RP:** The fruit fly is a great model for the study of obesity and T2DM, as the components of the IRS pathway are well conserved in this model organism. Like humans, flies raised on a high-fat diet develop a diabetic phenotype. Furthermore, the fruit fly has a short generation time and lifespan, low maintenance costs and a wide range of genetic tools, which allow temporal and spatial expression of the target genes. However, whilst flies and humans share many genetic and physiological similarities, ultimately the fly is not a mammalian model.

**YY:** Whilst modeling disease in flies could provide insights into the molecular mechanisms of Tribbles, it is important to confirm these observations in humans. That is why we used Mendelian randomization, a computational technique that mimics a clinical trial, to test the effect of Tribbles in humans. During a clinical trial, participants are randomly allocated into different groups. Hypothetically, one group would receive a drug that modulates Tribbles expression, while the other group would get a placebo. Similarly, we acquire genetic variations from birth, and some of these variants can affect the expression of Tribbles. Here, we used genetic variations in the Tribbles gene that can affect either the function or the expression of Tribbles to investigate its impact on human health. One limitation of our method is the uncertainty of the specific impact of each of these Tribbles mutations. We know that rs2295490 leads to a change in the amino acid sequence and alters the function of the Tribbles protein. For the other genetic variations, we found possible hints on how it might impact Tribbles expression, but these hypotheses still need to be confirmed experimentally. Taken together, our results suggest that Tribbles could be a regulator of key metabolic processes and highlight the importance of genetic variations linked to Tribbles for further research.

### What has surprised you the most while conducting your research?

**RP:** I was most surprised by how useful the fruit fly was as a model when studying metabolism and what a great hypothesis-generating platform it is. Another positive surprise was the always helpful community of *Drosophila* researchers.

**YY:** The scientific community researching flies is very friendly. For example, we wanted to test the effect of the rs2295490 mutation in flies. Prof. Dobens' group previously genetically engineered these flies, and they kindly provided us with the samples when we needed them. What also surprised me was the possibility of modeling complex genetic causes in humans. It is well known that single genetic variations could be linked to gene expression. It would be interesting to investigate whether genetic variations that cause a change in gene expression could also be linked to diseases. Importantly, this opens avenues for understanding the effect of that variation on gene expression. Understanding the effect of genetic variations is important for personalized medicine and also age-related diseases. For example, if we confirm that a particular genetic variant causes higher Tribbles expression, doctors could prescribe drugs to inhibit Tribbles specifically to those patients. Additionally, we acquire mutations through ageing and with different diseases like cancer and Alzheimer's disease. A better understanding of how genetic changes in our cells can directly affect important aspects of our lives like sleep and weight would also contribute to more precise treatments.

Disease Models & Mechanisms (2023) 16, dmm050220. doi:10.1242/dmm.050220

**What do you think is the most significant challenge impacting your research at this time and how will this be addressed over the next 10 years?**

**RP:** Organ functions are interdependent; therefore, it is important to understand how different tissues communicate with each other. Thus, with the increasing awareness of faulty interorgan signalling in disease, I think the most significant challenge will be to develop systems to study how miscommunication between different organs may result in the loss of organismal homeostasis.

**YY:** I believe that, very soon, the scientific community will have generated data on genetic variations linked to the levels of genes, proteins and metabolites in different cell types in different tissues. These would be precise genetic variants linked to the gene, protein or metabolite level in a particular cell type of a specific organ area. With this information, we could understand how molecular mechanisms in a specific group of cells could cause a disease or behaviour. Currently, it is possible to measure the levels of mRNA in single cells in a given tissue area. However, it is harder to do this at the proteome and metabolome levels. I am very optimistic that, with more technological advances, scientists can acquire these high-specificity data at a very large scale and thus compute the genetic variations that control person-to-person variations in these subtle changes. This will also lead to a better understanding of how molecules, cells, organs and organisms function. The next step, of course, would be to understand how these genetic variants could influence changes at the tissue-specific single-cell level. Exciting science!

> **“[…] life sciences require a lot of time and personal commitment, but opportunities to finance these projects are becoming more and more scarce.”**

**What changes do you think could improve the professional lives of scientists?**

**RP:** I think the lack of work–life balance, financial support and a stable career trajectory are some of the key challenges that young scientists face at the moment. There are many early-career scientists motivated to pursue a career in science; however, without the appropriate system to support them, it is hard to achieve this goal.

**YY:** I have really enjoyed my scientific path so far. If there was something that could still be improved, it would be the availability of better career opportunities. For instance, life sciences require a lot of time and personal commitment, but opportunities to finance these projects are becoming more and more scarce. I believe that a better societal effort should be dedicated to understanding how to stay healthy. High-quality research requires a lot of dedication, both in terms of time and funding. I therefore think that it’s important that our society allocates more talent and funding to research and applies evidence-based insights to improve public health.

**What’s next for you?**

**RP:** For my postdoctoral studies I wanted to focus on translational neuroscience and work on projects closer to patients, which led me to the postdoctoral research position at the UCL Drug Discovery Institute (DDI). The DDI is discovering glial therapeutics for Alzheimer’s and other neurodegenerative diseases. I am currently developing glial functional assays as part of a multidisciplinary team of translational scientists including medicinal and computational chemists, screening pharmacologists and neuroscientists.

**YY:** At the moment, I’m writing up my thesis. In the future, I’d be very keen to work on cool science techniques like using cryogenic electron microscopy to visualize the structures of molecules *in situ* or measuring proteins or metabolites in individual cells using mass spectrometry. I’m also very interested in developing and applying computational methods like causal inference or graph machine learning to find the causes of age-related diseases.

**Reference**

**Popovic, R., Yu, Y., Leal, N. S., Fedele, G., Loh, S. H. Y. and Martins, L. M.** (2023). Upregulation of Tribbles decreases body weight and increases sleep duration. *Dis. Model. Mech.* **16**, dmm049942. doi:10.1242/dmm.049942