FIGHT MND.

Early-Career FightMND Research Fellow.
DR REBECCA SAN GIL

Research.
GENOME-WIDE CRISPR SCREENS TO REVEAL REGULATORS OF TDP-43 AGGREGATION AND TOXICITY IN MND


Dr Rebecca San Gil

Who are you and where do you work?

My name is Rebecca San Gil and I am excited to be Fight MND's first Early-Career Research Fellow awarded in 2019. I conduct my research at the Queensland Brain Institute, University of Queensland, in the Neurodegeneration Pathobiology Laboratory.

Summarise your research experience and background?

My PhD at the Illawarra Health and Medical Research Institute studied the role of the cellular heat shock response as a first responder to protein aggregation and neuroinflammation in neurodegenerative diseases. I showed that protein aggregation impairs or evades the detection of the heat shock response. This means that neurons likely can't protect themselves from a major pathology that drives neurodegenerative diseases. During my PhD I undertook an Endeavour Research Fellowship at the Sobell Department of Motor Neuroscience and Movement Disorders, University College London. There, I developed an intensive research focus on MND and acquired skills in a range of techniques to study the pathogenesis of disease in motor neurons and other cell types that support motor neuron health and survival (astroglia and microglia). After completing my PhD in 2018, I was recruited by the Queensland Brain Institute to continue research into advancing our understanding of the molecular triggers of MND and identifying therapeutic targets in motor neurons, the brain, and spinal cord.

What got you interested in researching on MND?

I had the privilege to work alongside Prof Yerbury, who is a world leading expert in protein homeostasis in MND, but is sadly also battling MND. I have witnessed firsthand the daily trials of living with MND and the persistence and dedication Prof Yerbury has shown. He is an exceptional role model and mentor and his journey has inspired me on my research path investigating and developing therapeutics to improve and extend the lives of patients with MND. Despite the many breakthroughs in recent years, I felt it was important to put the skills and techniques I had learnt to good use to better understand essential questions about the pathogenesis and strategies to treat MND more effectively.

What is your favourite scientific finding so far?

The discovery of CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) technology is my favourite finding so far. I think it is the versatility of CRISPR/Cas9 that makes it the greatest discovery of all time. CRISPR/Cas9 can be used to very precisely edit, knock-out, inhibit and activate genes. It is highly likely that CRISPR/Cas9 will be a key player in drug-target discovery for many human diseases in the future.

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Can you update us on the research you are currently pursuing?

I am very excited to be starting a new project using human genome-wide CRISPR screening to identify genes and proteins that regulate protein aggregation in MND. Aggregation of the TDP-43 protein is associated with toxicity that causes the death of motor neurons in 97% of MND cases. One potential therapeutic strategy is to target and prevent this toxic process of TDP-43 clumping, however, the mechanisms involved in driving TDP-43 aggregation remain unclear. This project will use revolutionary new gene editing technology to scan all 20,000 genes in the human genome, to identify the mechanisms in cells that can trigger or stop TDP-43 aggregation. This will reveal new targets that will be tested for therapeutic potential in preclinical models of MND, with the ultimate objective of identifying future avenues to treat people living with MND.

What excites you most about the potential to eradicate TDP-43 aggregation?

Many lines of evidence point to protein aggregation being one of the initial molecular dysfunctions that lead to neuroinflammation and neurodegeneration in MND. I strongly believe that by understanding and inhibiting aggregation, the first step, we can stop disease onset and progression. This concept will be directly tested as part of this Fellowship. In addition, TDP-43 aggregation is a key unifying pathology common to 97% of MND cases. Therefore, targeting TDP-43 aggregation might provide us with the insight to treat nearly all cases of MND.

What difference will this fellowship make to your research?

This FightMND Fellowship is an excellent opportunity for continued development of my professional and experimental skillset. This project will be conducted in collaboration with Prof Naomi Wray FAA (UQ), Dr Shyuan Ngo (UQ), Prof Aaron Gitler (Stanford), A/Prof Todd Cohen (University of North Carolina) and will represent an invaluable opportunity to learn from world leaders in MND research. In addition, this project will generate a career's-worth of drug targets to pursue and enable me to build on my existing skillset by developing skills in CRISPR/Cas9 gene editing, next generation sequencing, and bioinformatic analysis pipelines. Ultimately, this fellowship is a career-making opportunity and excellent stepping stone for me to continue to build a career in research with a focus on finding new therapeutics to treat or cure people living with MND.

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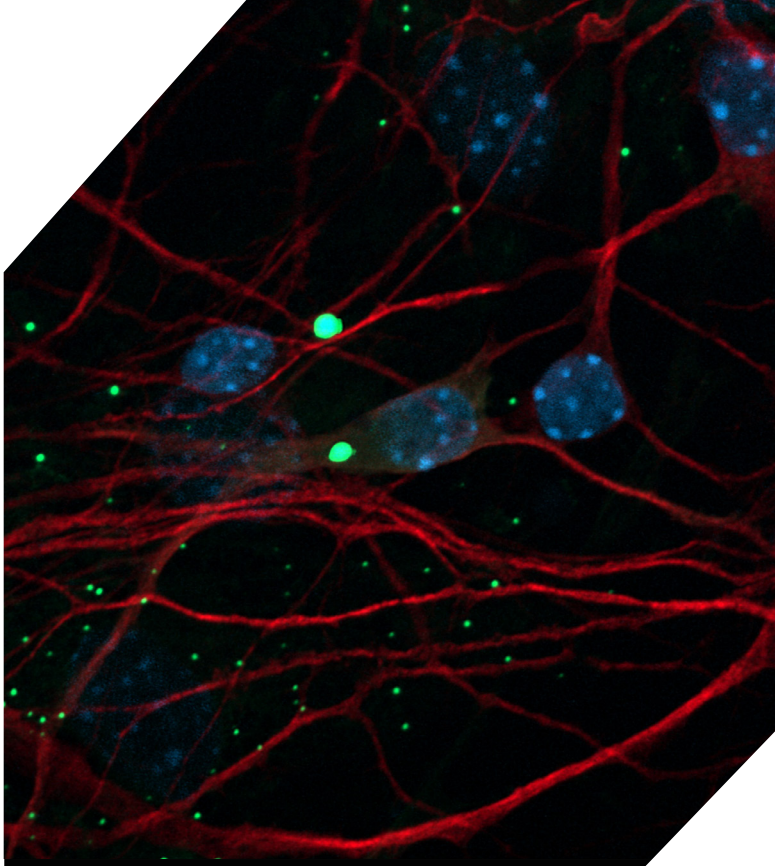

Mouse primary spinal cord neurons (red) transduced with lentiviral particles to over-express aggregation-prone TDP-43 (GFP-tagged; green) in the cytoplasm.

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One of the first events, at the molecular level, which leads to disease onset in all sporadic (and most familial) cases of motor neuron disease (MND) involves the build-up of a protein called TDP-43 in motor neurons. TDP-43 proteins stick together to form small toxic clumps. Over time these small clumps become the building blocks of larger protein inclusions that are strongly associated with the death of motor neurons in MND and are clearly visible under a microscope. Therefore, one potential therapeutic strategy is to prevent this toxic process of TDP-43 clumping. Unfortunately, there is currently a limited understanding of how TDP-43 clumps and why it is toxic.

The primary aims of this Fight MND Fellowship are to identify biochemical pathways that enhance or inhibit TDP-43 clumping and its associated neurotoxicity, and to conduct preclinical testing of promising regulators of TDP-43 clumping in a validated TDP-43 mouse model of MND. This project will use revolutionary gene editing technology to scan all 19,000 protein-encoding human genes in the human genome to identify the genes that modulate TDP-43 clumping. The innovative gene editing technology that enables us to scan every human gene is cutting-edge, and the experimental strategy that will be implemented would not have been possible even a few years ago.

The research performed will be crucial for identifying mechanisms that regulate the processes that cause MND. There will be a strong focus on validating the preclinical value of these findings in studies using a mouse model of TDP-43-associated MND, on the path towards more effective treatments for people living with MND.

OUTCOMES:

1. To conduct human genome-wide CRISPR/Cas9 gene knockout and activation screens to identify enhancers and inhibitors of TDP-43 aggregation and its associated neurotoxicity.
2. To establish the mechanisms of action of the strongest regulators of TDP-43 aggregation in neuroblastoma cell lines, and motor neurons derived from preclinical MND models and MND patients.
3. To perform preclinical testing of promising regulators of TDP-43 aggregation in a validated TDP-43 mouse model of MND, using gene delivery to the central nervous system.