Generation of Humanised STX6 Overexpression Mice to Study Prion Disease Genetic Risk and as a Model for Therapeutic Intervention

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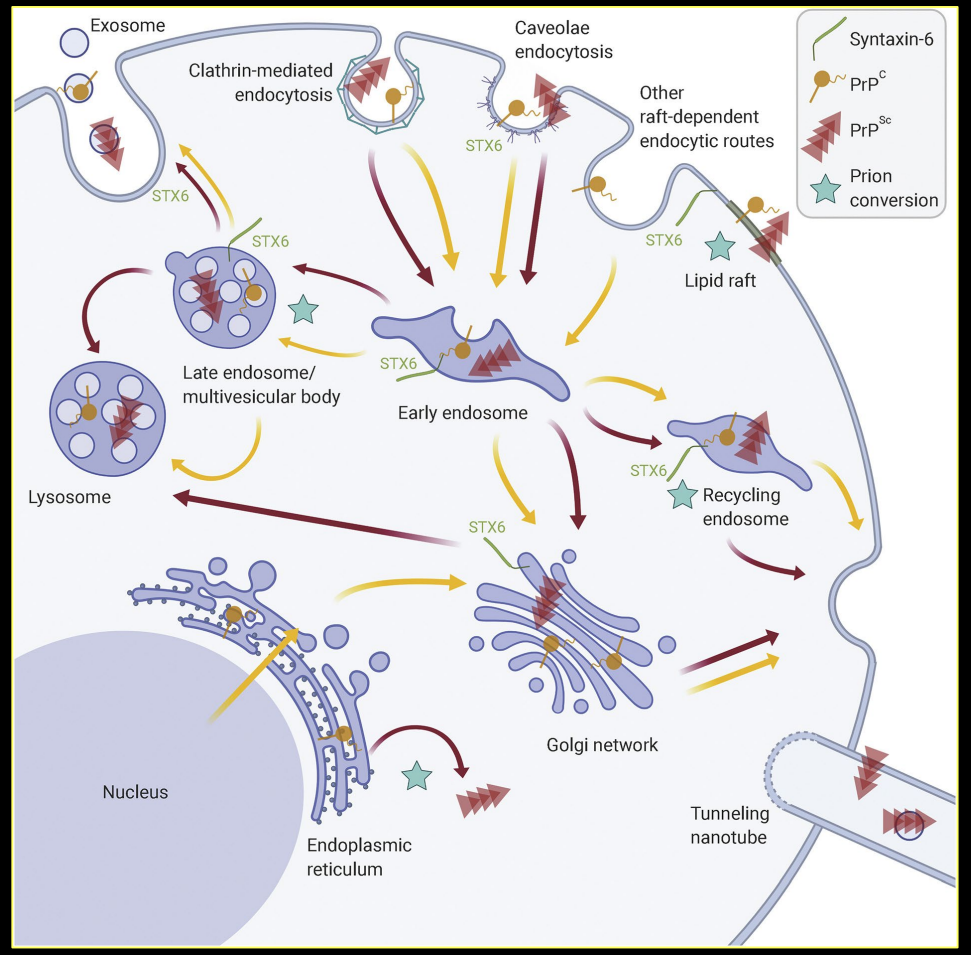
20th July 2024

Syntaxin-6 (*STX6*)

- **What is Stx6?**
  - A protein that regulates transport of proteins and other molecules around cells
  - e.g. helps to transport cargo for recycling or degradation, or delivery of cargo to different parts of the cell



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  - People with the risk variant → 16% higher chance of developing sCJD & produce higher levels of STX6

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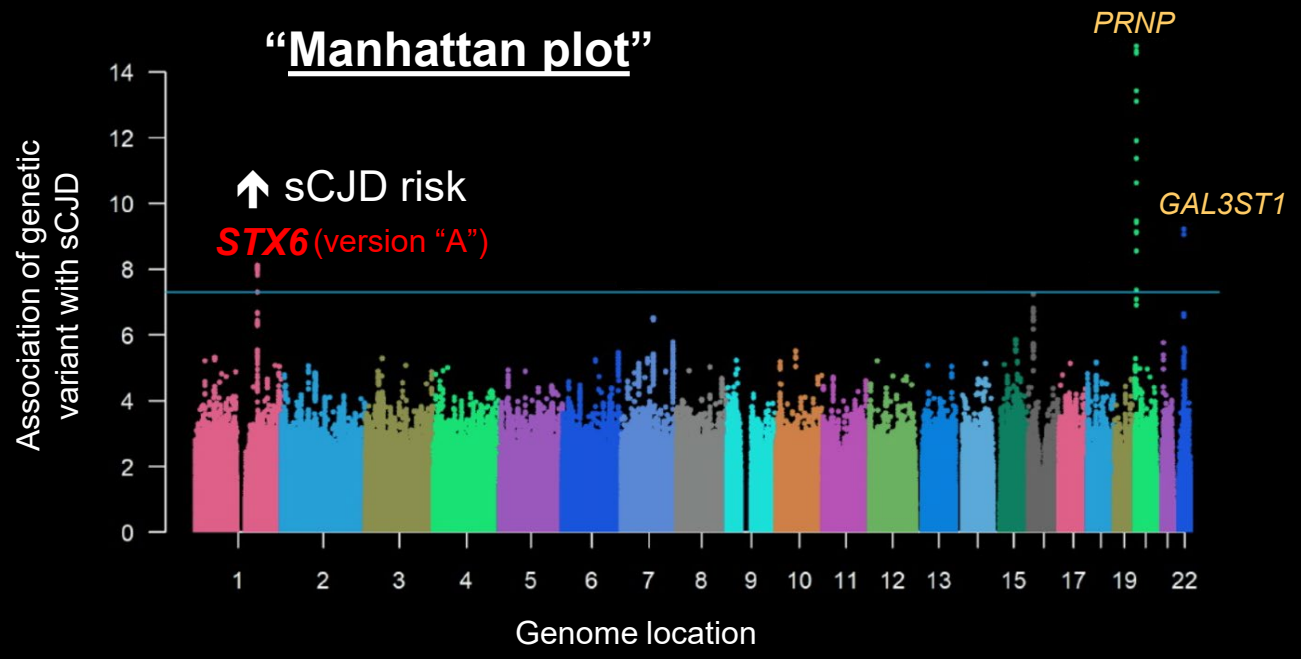
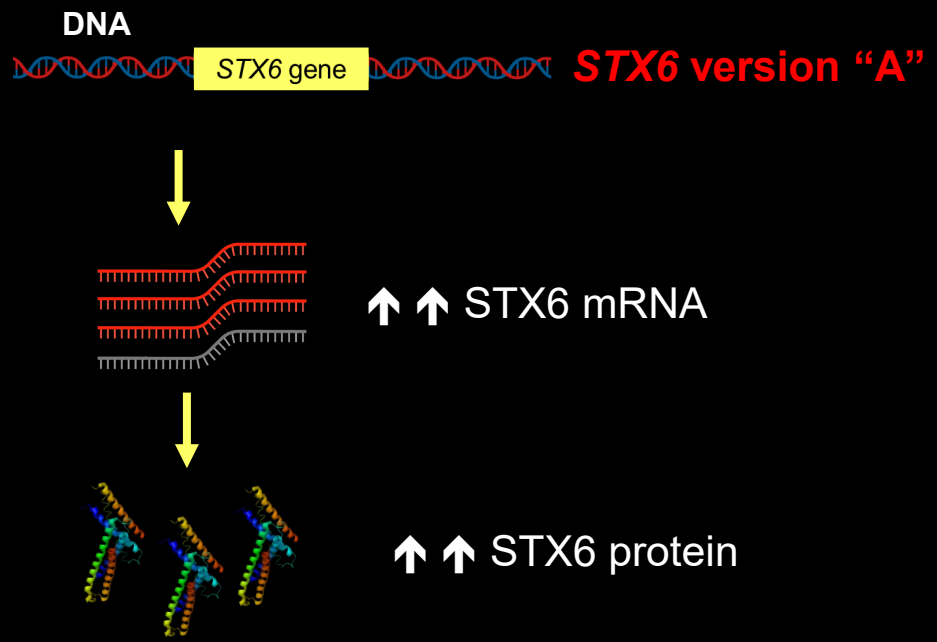


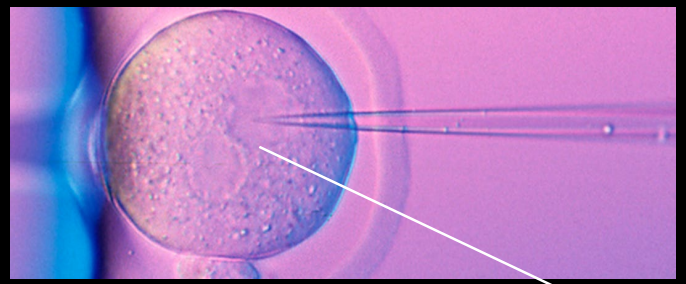


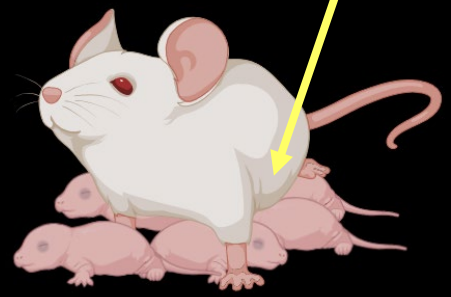
Mouse genetics

- **Why use mouse models**
  - 98% of genes in humans are also present in mice, and most perform the same function
  - Mouse models of prion disease recapitulate hallmarks of the human disease, in a short timeframe
  - We can relatively easily genetically modify mice to model human genetics findings

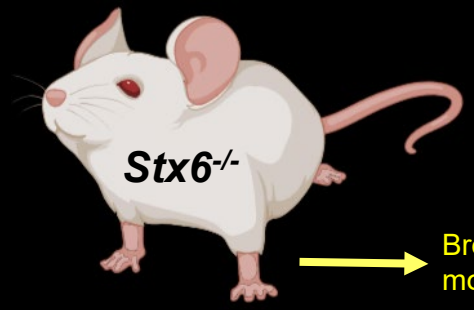
Stx6 "knockout" mice (*Stx6*$^{-/-}$)

Single cell embryo microinjection





Embryo re-implantation
& birth of modified mice



Breed to maintain
modified line

cell nucleus

Gene deletion via CRISPR/Cas9 "molecular scissors"

DNA

Stx6

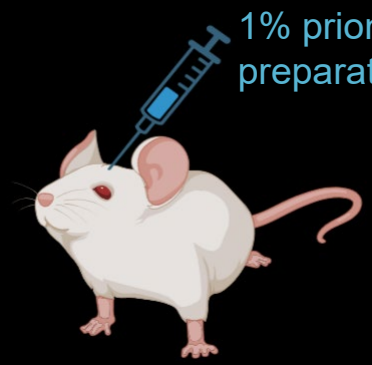
Stx6 "knockout" mice (*Stx6*$^{-/-}$)

- **Removal of *Stx6* has no meaningful impact in mice infected with a high dose of prions (1%)**

- *Stx6*$^{-/-}$ mice develop prion disease within a similar timeframe to controls
- Similar levels of infectivity throughout disease
- Similar changes in the brain

Stx6 "knockout" mice (*Stx6*$^{-/-}$)

- **What could we be missing?**



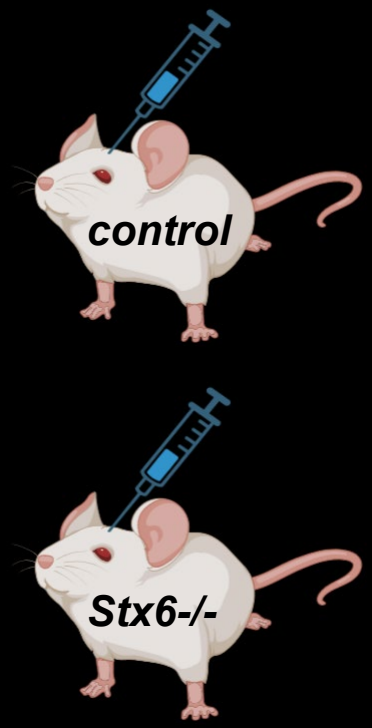
- 1% is a very high dose!
  - 100% of mice get disease
  - We can model later disease stages very well
  - Not ideal to investigate disease initiation

?	?	No effect of Stx6-/-	
Spontaneous misfolding & formation of first prion	Establishment of prion seeds & early replication	Replication and spread throughout brain	Neurotoxicity

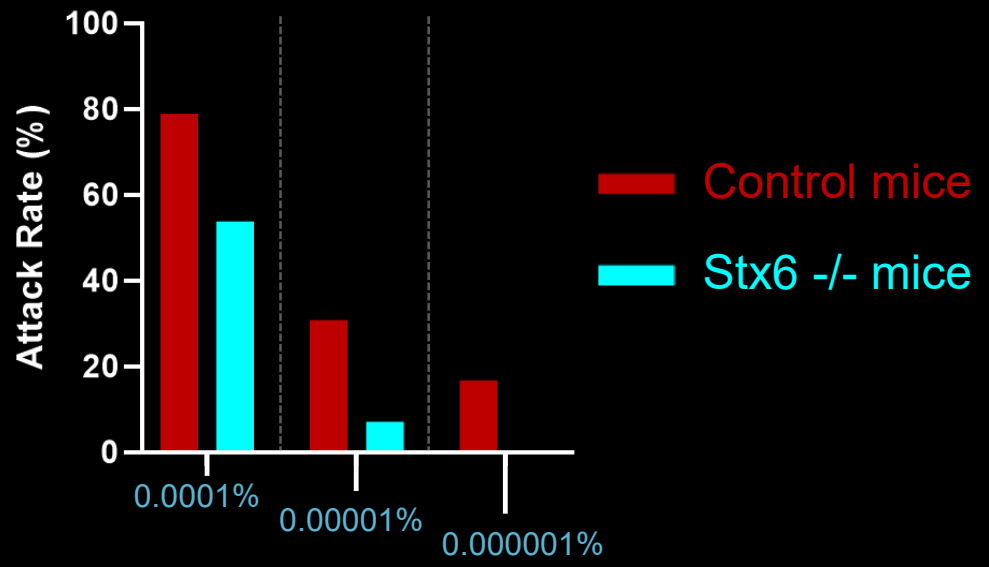
Prion disease stages

Stx6 "knockout" mice (*Stx6*-/-)

- **Serial dilution experiment: dilute prions up to 1 million x, before infection**
  - At very low doses we can measure how Stx6 impacts establishment of early disease
  - How many mice become infected as we dilute down ("attack rate")?



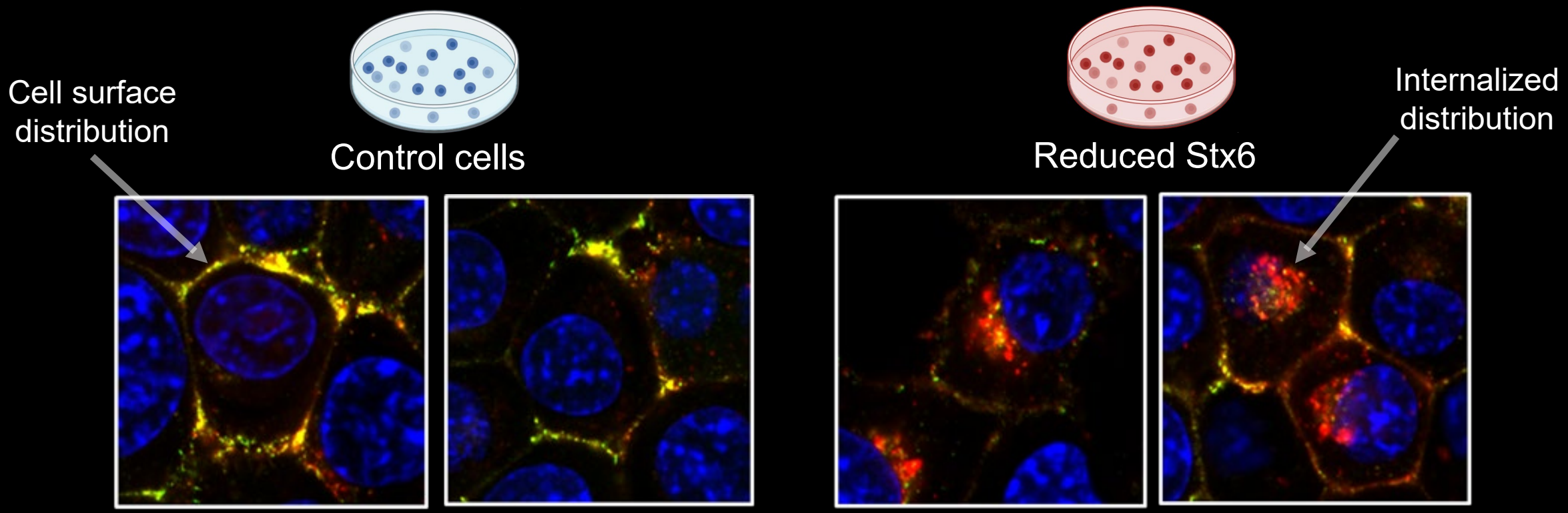
0.1,
0.01,
0.001,
0.0001,
0.00001, or
0.000001% prion preparation



- Stx6 lowering is protective at very low doses of infection
  - We think the effect acts <u>early</u> in disease

Stx6 cell models

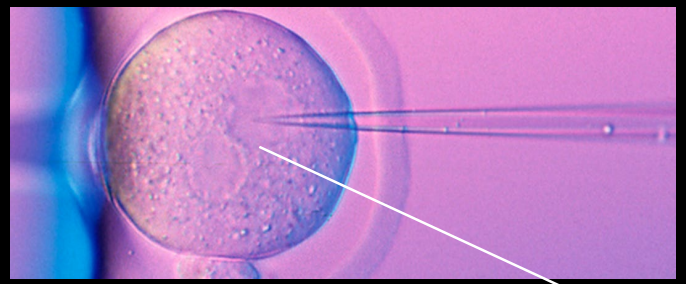
- **We can also change Stx6 levels in cells in a dish, and measure the effect on prion infection**



- Altering Stx6 changes the distribution of prion protein
  - Consistent with a role in cell transport

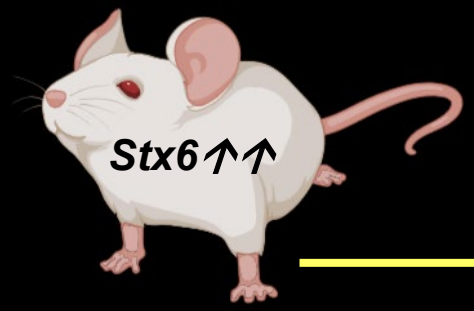
Stx6 overexpression mouse model

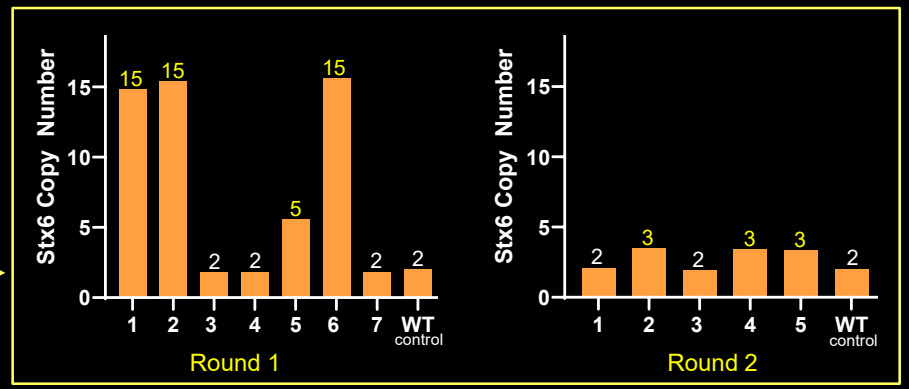
Single cell embryo microinjection



Adding extra copies of the Stx6 gene to mimic the risk affect

Embryo re-implantation & birth of modified mice





- New lines generated with 3x, 5x, & 15x copies of Stx6

Summary and Conclusions

- Genetic variation in the gene Syntaxin-6 (STX6) increases levels of the gene and protein and increases risk for developing sCJD.

- STX6 is involved in trafficking proteins and other molecules within cells.
- Removal of mouse Stx6 has a modest protective effect – most prominent when mice are infected with a very low concentration of prions, indicating the effect may lie with how brain cells deal with very early infection.
- Cell-culture studies show a change in cell distribution, consistent with a role in cell transport.
- STX6 lowering does not appear to be a promising candidate for treating established prion disease. Protective effects during very early disease stages require further investigation to better understand the cellular mechanisms.
- We have engineered new mouse models for studying how increased Stx6 levels affects prion disease, to mirror the effect of the risk variant in humans, and to compliment our mouse studies investigating Stx6 removal.

Acknowledgements

MRC Prion Unit
UCL Institute of Prion Diseases

Lizzie Hill

Simon Mead lab

Cunningham lab:
Andy Tomlinson
Tatiana Jakubcova
Shyma Hamdan

Klöhn lab

Biological services facility

Histology facility

The Michael H. Cole Memorial Research Grant,
contributed by Jeanne Cole

The Daniel L. Dolgin Celebration Grant,
contributed by his family

The Fred Glavan/Lee Gallagher Family Memorial Grant,
contributed by the Glavan and Gallagher families

The Ross Melamed Memorial Grant,
contributed by Olivia Melamed and family and friends

The Andy Lewis Memorial Grant,
contributed by his daughters, extended family, and friends

The José A. Piriz and Sonia E. Piriz Memorial Research Grant,
contributed by Karla Piriz and Lauren

The Tom Stivison Memorial Research Grant,
contributed by Sandra (Cookie) Stivison

The Strides for CJD Grant,
contributed by the Families of the CJD Foundation

Medical Research Council