Discovery and functional genetics analysis of human prion disease modifiers

…and its intersection with Alzheimer's disease and related dementia disorders

Prof Simon Mead

National Prion Clinic, UCLH, London, UK

MRC Prion Unit at UCL

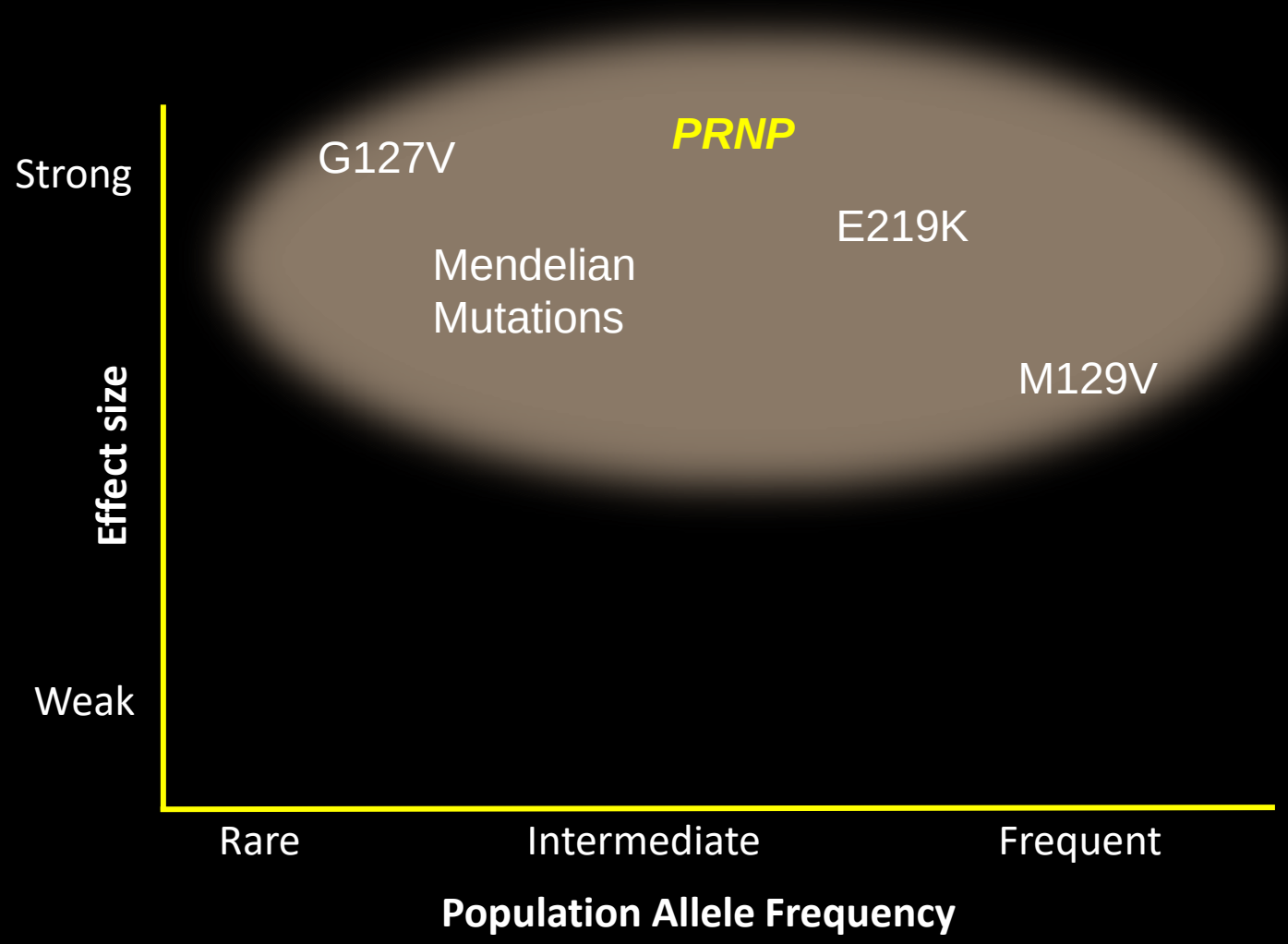
Institute of Prion Diseases

Summary

An update on human genetics and prion diseases from a collaborative group of clinical researchers led by MRC Prion Unit at UCL, London

- Strong genetic effects in prion disease and ADRD
- Genome wide association study of sporadic CJD and extensions
- Multi-omic analysis of genetic risk factors in CJD and AD
- Pathways and gene prioritisation
- Direct correlation of AD and CJD
- Correlation with other dementia disorders - PSP
- Overlap 1: intracellular trafficking - STX6
- Overlap 2: lipid metabolism – sulfatides/premetabolites

Genetic architecture of human prion diseaes



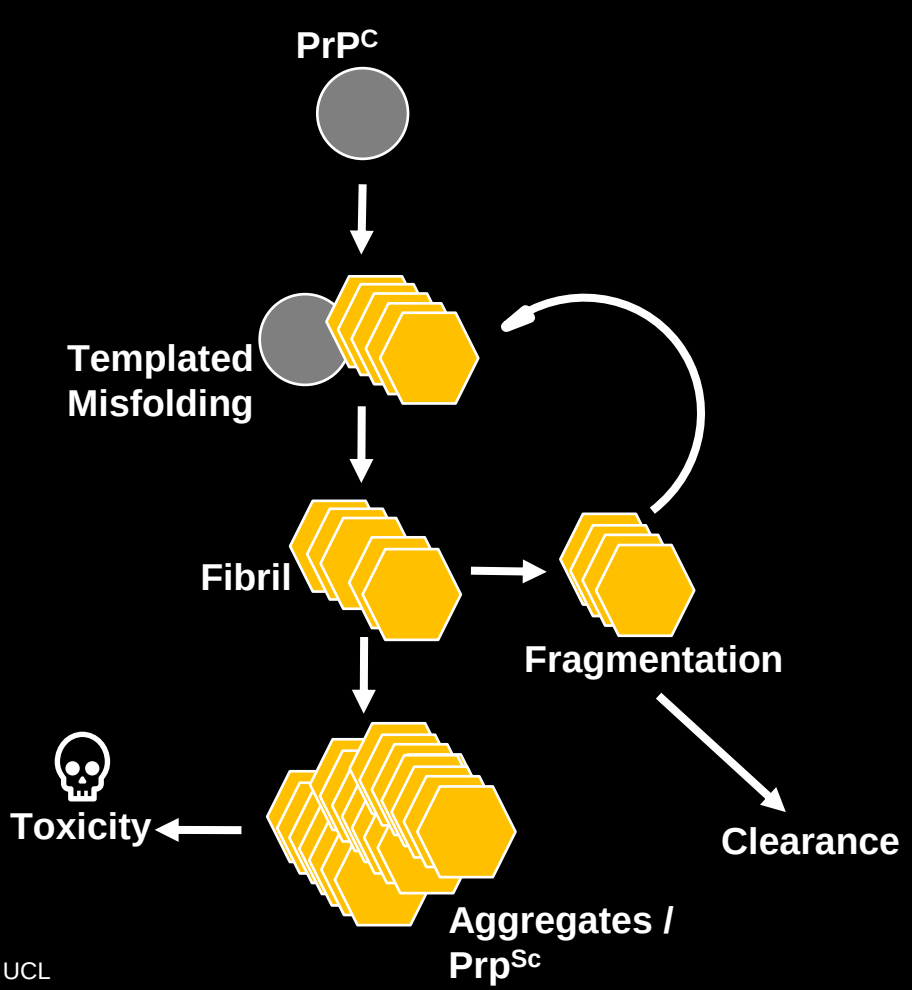
- Myth of infectious disease most prion disease is sporadic or inherited ~AD
- Certain amino-acid altering variants of PrP massively alter risk of disease
- Background risk CJD ~1:5000 to close to certainty, increase 10-100x, increase moderately, protect modestly, or offer complete resistance
- Comparable to *APP* variants in familial AD

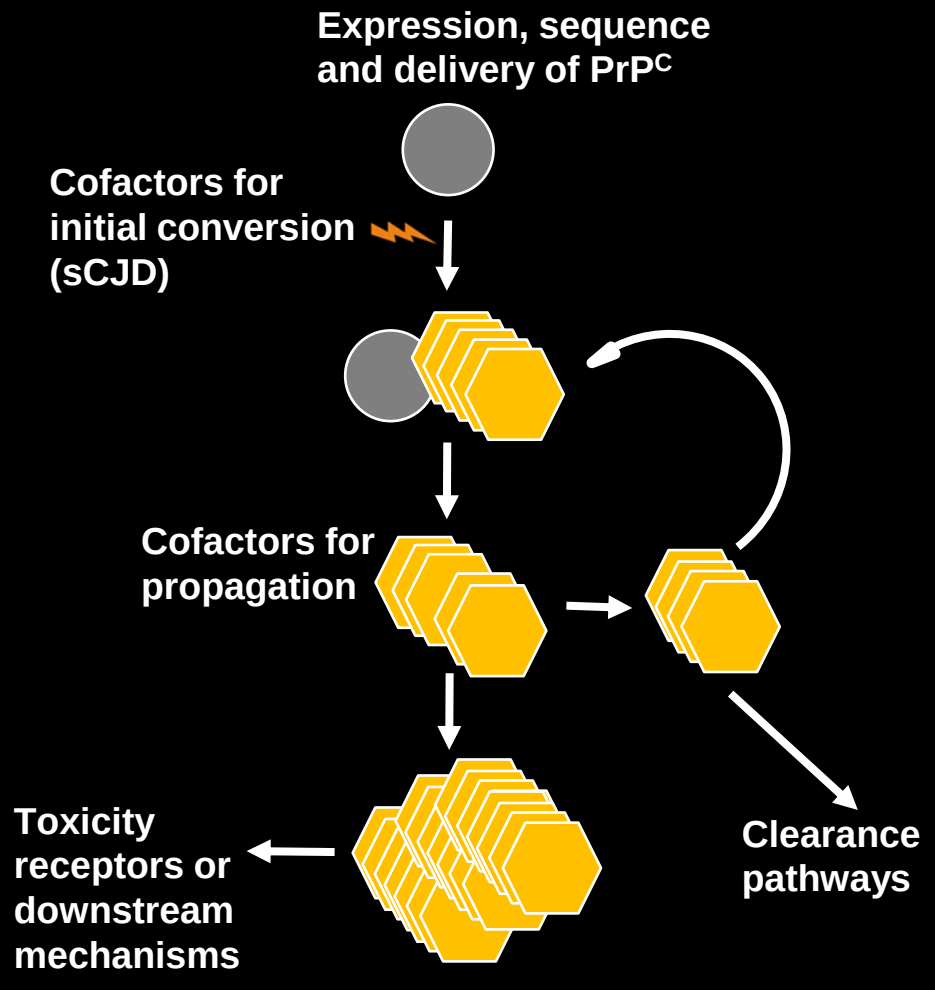
Prion mechanisms – which are shared in prion-like conditions?

Simple model no partners

or

Complex model, crucial molecular partners





Genome wide association study

Why do we want to do it?

- Aside from Randomised Controlled Clinical Trials best way to discover ***causal*** mechanisms in humans

- Fundamental origin of the most common human prion disease sCJD is uncertain

- May answer questions about shared aetiology prion and prion-like

- Potential to discover genes, cell types, broader mechanisms of human disease that hitherto were entirely unpredicted

- We know PrP is *the* target and drug pipeline expanding, but would like more than one target, genetics may also inspired therapeutic mechanisms at a target

- Human genetically-inspired drug targets are in general more likely to succeed in development, true for both Mendelian linked loci, and weaker, GWAS loci

Genome wide association study

DNA from Patients diagnosed with at least probable CJD

What is a typical GWAS?

Data or DNA from Healthy population control individuals

660,000 Single nucleotide polymorphisms in each case

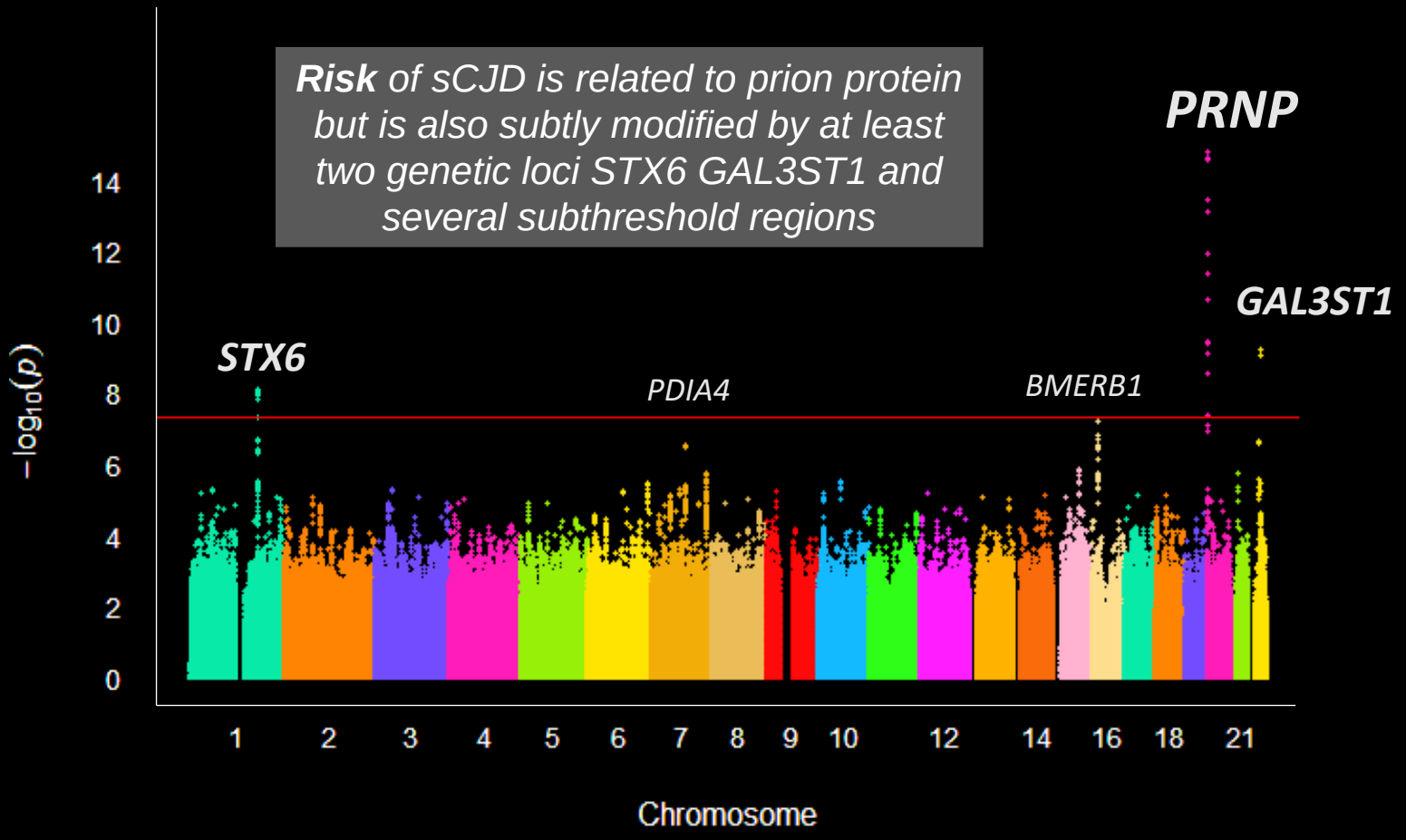
Quality Control

Imputation using huge population panels to fill in gaps 6-10M SNPs

Statistical comparison of groups

660,000 Single nucleotide polymorphisms in each control individual

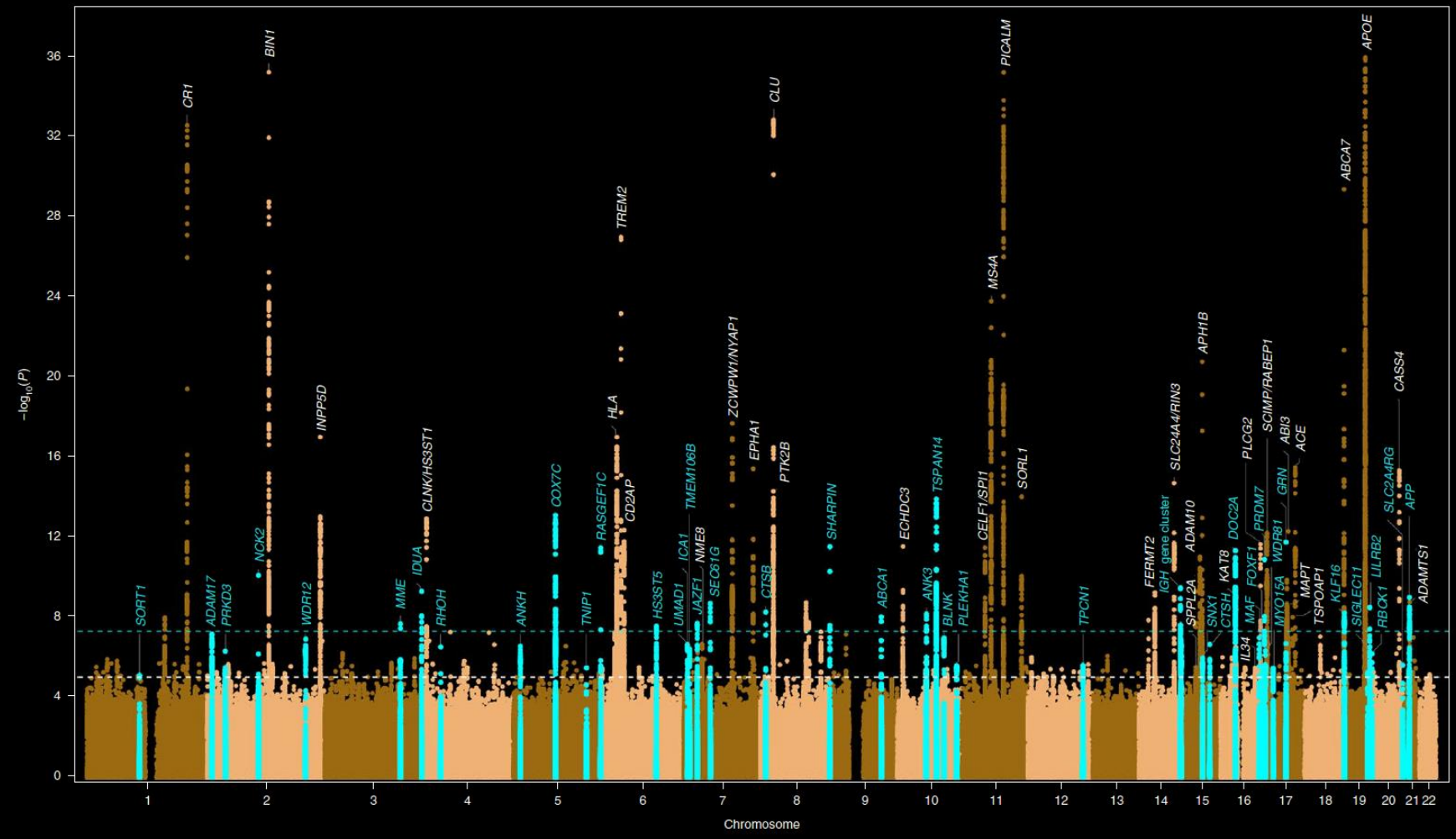
GWAS – 2020 CJD discoveries and extensions



- **5,208 clinically or pathologically diagnosed cases and 13,569 controls**
- **3 risk loci, 2 others**
- **Pathway analysis reveals no significant gene sets enrichment**
- **Cell type specific expression reveals oligodendrocyte expressed genes (*STX6* eQTL, *GAL3ST1*)**
- **No non-*PRNP* determinants of Age of onset or Duration and no genetic correlation between these phenotypes**

Jones et al. Lancet Neurology 2020; Hummerich et al. PLOS One 2024

GWAS –Alzheimer's disease Bellenguez, Kucukali et al. 2022 (*Nat Genet*)



- 111,326 clinically diagnosed/'proxy' AD cases and 677,663 controls
- 75 risk loci
- Pathway analysis reveals gene sets related to amyloid, tau, lipids, endocytosis and immunity (macrophage and microglial activation)
- Cell type specific expression reveals microglial expressed genes enriched

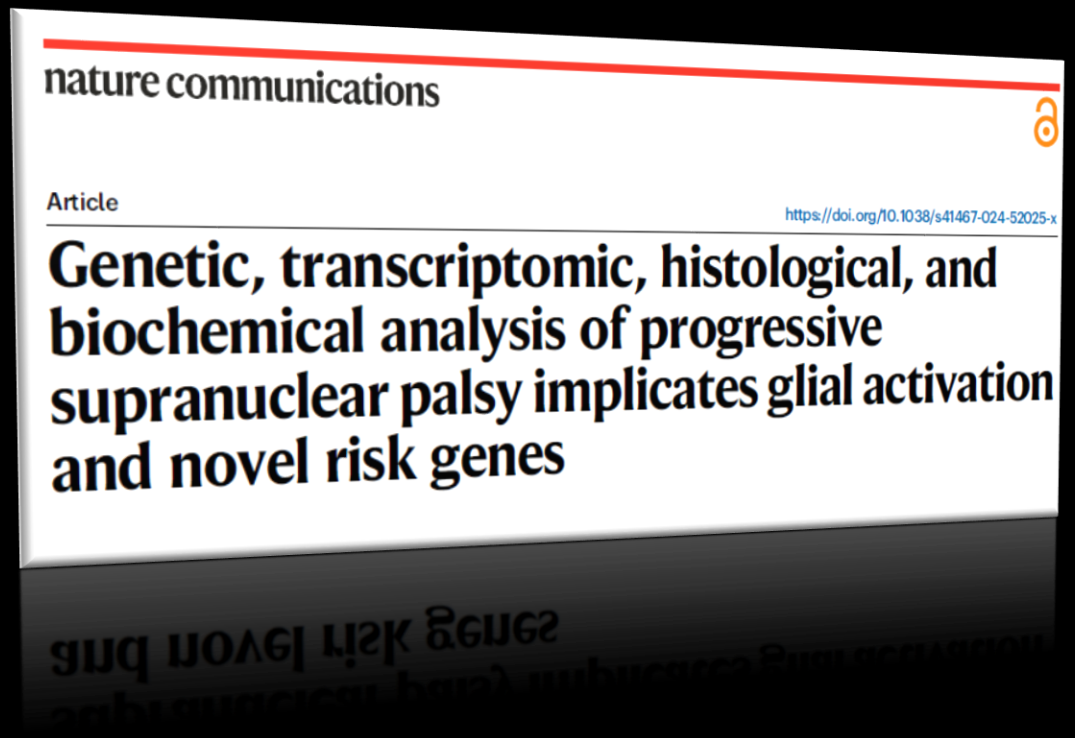
GWAS – Genetic Correlation between CJD, AD and other dementias

- Use GWAS data to ask how heritable and to compare traits ie how genetically correlated
- Linkage Disequilibrium Score Regression
- AD vs CJD – Correlation 0.31 (se=0.19) P=0.10

(similar for other dementia disorders meaning *overall* each disorder has its own set of risk genes)

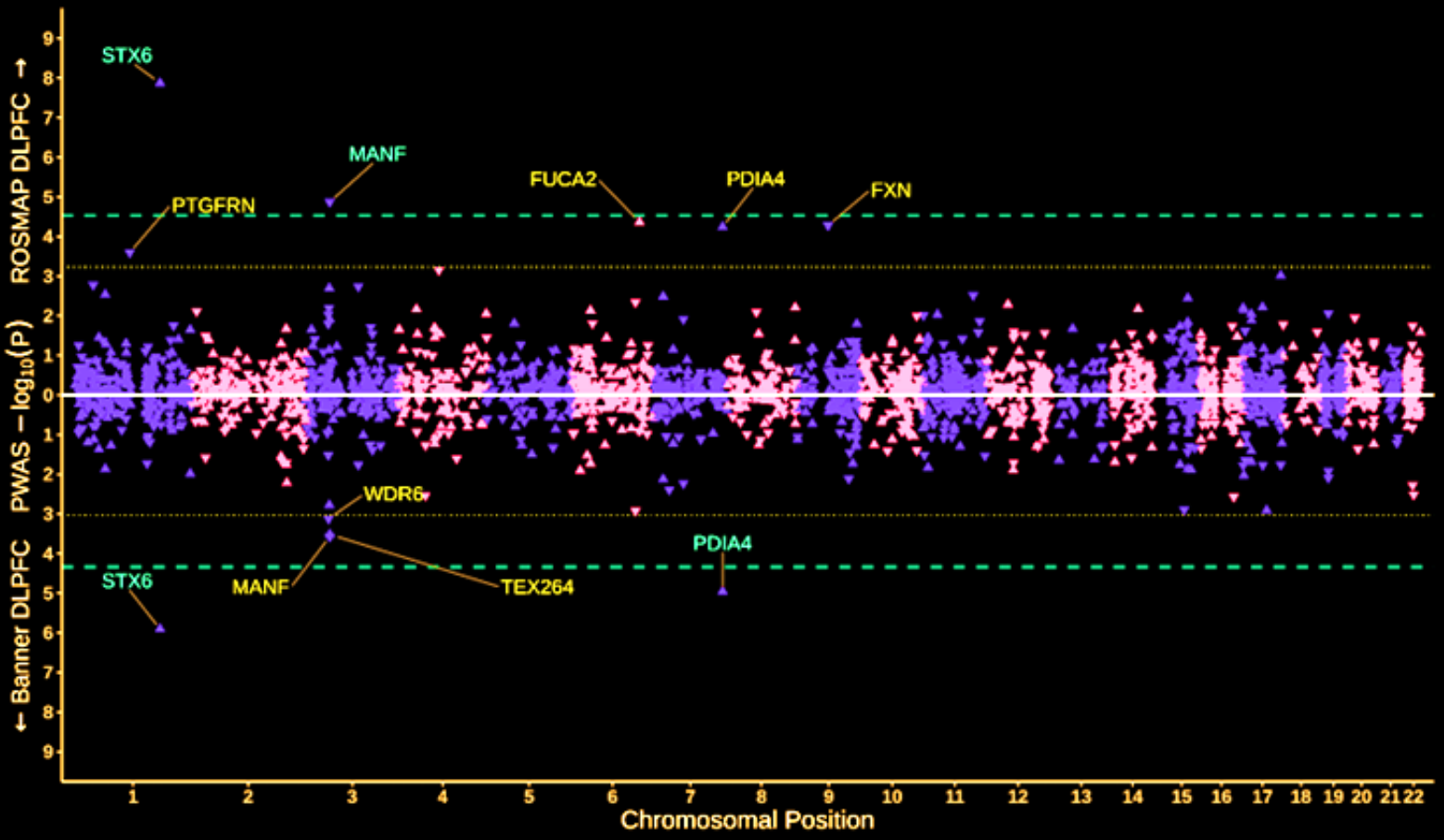
…but story different when look at individual genes

GWAS – Genetic Correlation between CJD, PSP



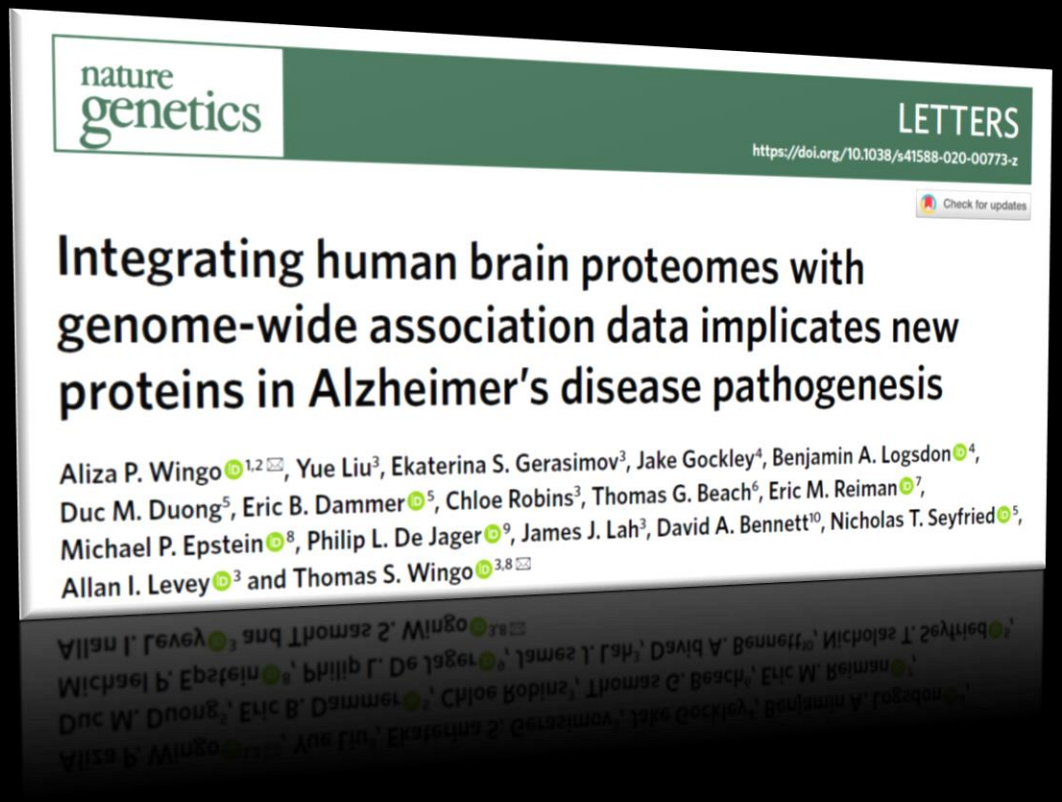
- Progressive Supranuclear Palsy genetic risk loci: *MAPT, MOBP, STX6, RUNX2, SLCO1A2, C4A*
- Proposed index SNP intronic rs3789362-A increases *STX6* expression by modifying an oligodendrocyte-specific enhancer sequence
- In CJD we proposed index SNP rs3747957-A which is a synonymous transcript variant
- Two SNPs are 2161bp apart and in perfect correlation in European ancestries populations
- Almost certainly the same genetic risk effect

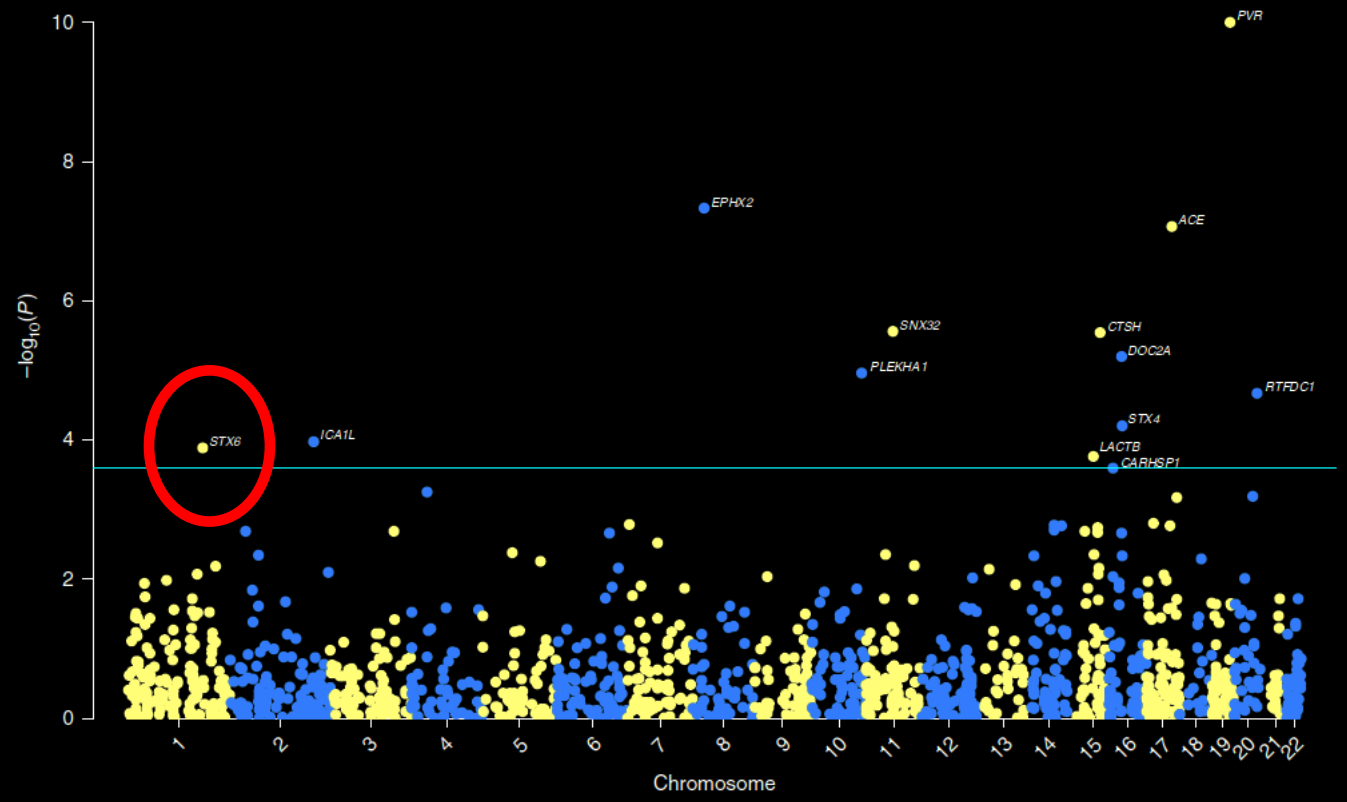
Transcriptome and Proteome wide association study



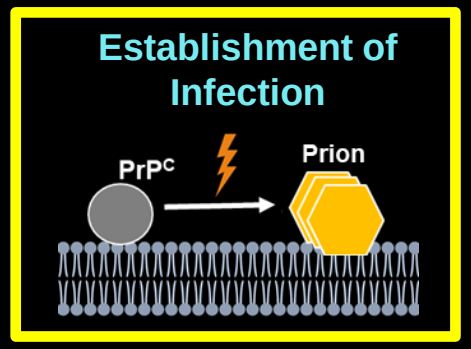
- Worked with second author of the Bellenguez et al. paper in AD using the same methods
- Model of transcript and protein expression based on shared brain bank and blood data (healthy people)
- Here looking at whether modelled levels of protein or transcript expression affect risk of CJD
- Two different frontal cortex series
- *STX6* is PWAS significant
- *PDIA4* protein also
- New protein product of *MANF* also
- Mesencephalic Astrocyte Derived Neurotrophic Factor

Transcriptome and Proteome wide association study



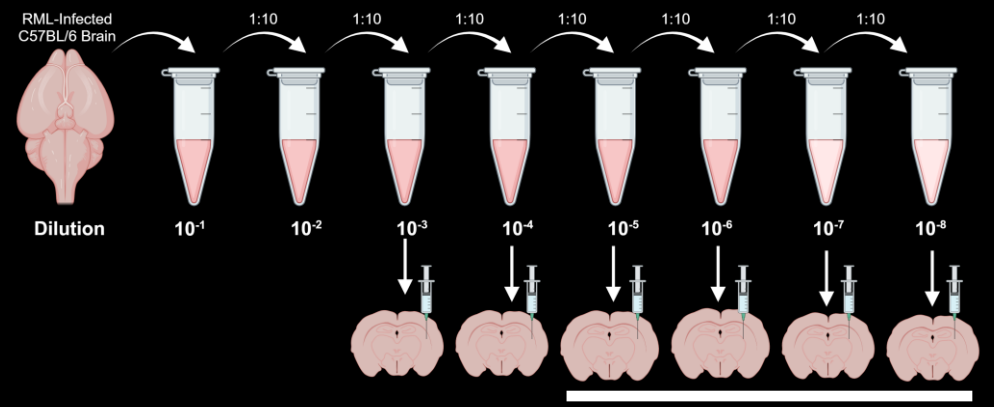


Assessing the Role of *Stx6* in the Initial Establishment of Infection *in vivo*



Study Design

Stx6$^{+/+}$ and *Stx6*$^{-/-}$ mice were infected with a titration series of RML prions.

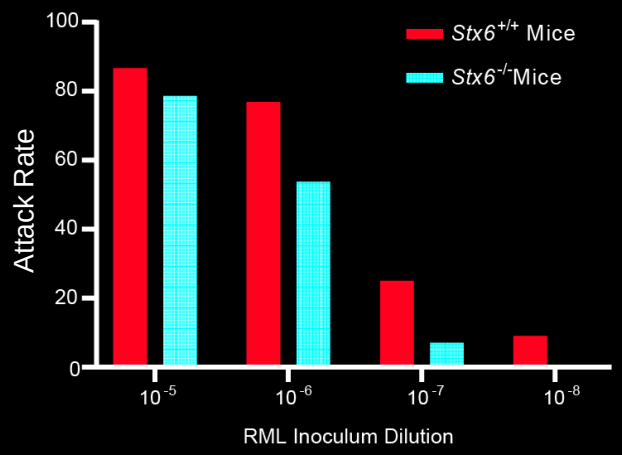


Focus = Doses with a Partial Attack Rate

Stx6$^{-/-}$ Mice are Less Susceptible to Prion Infection

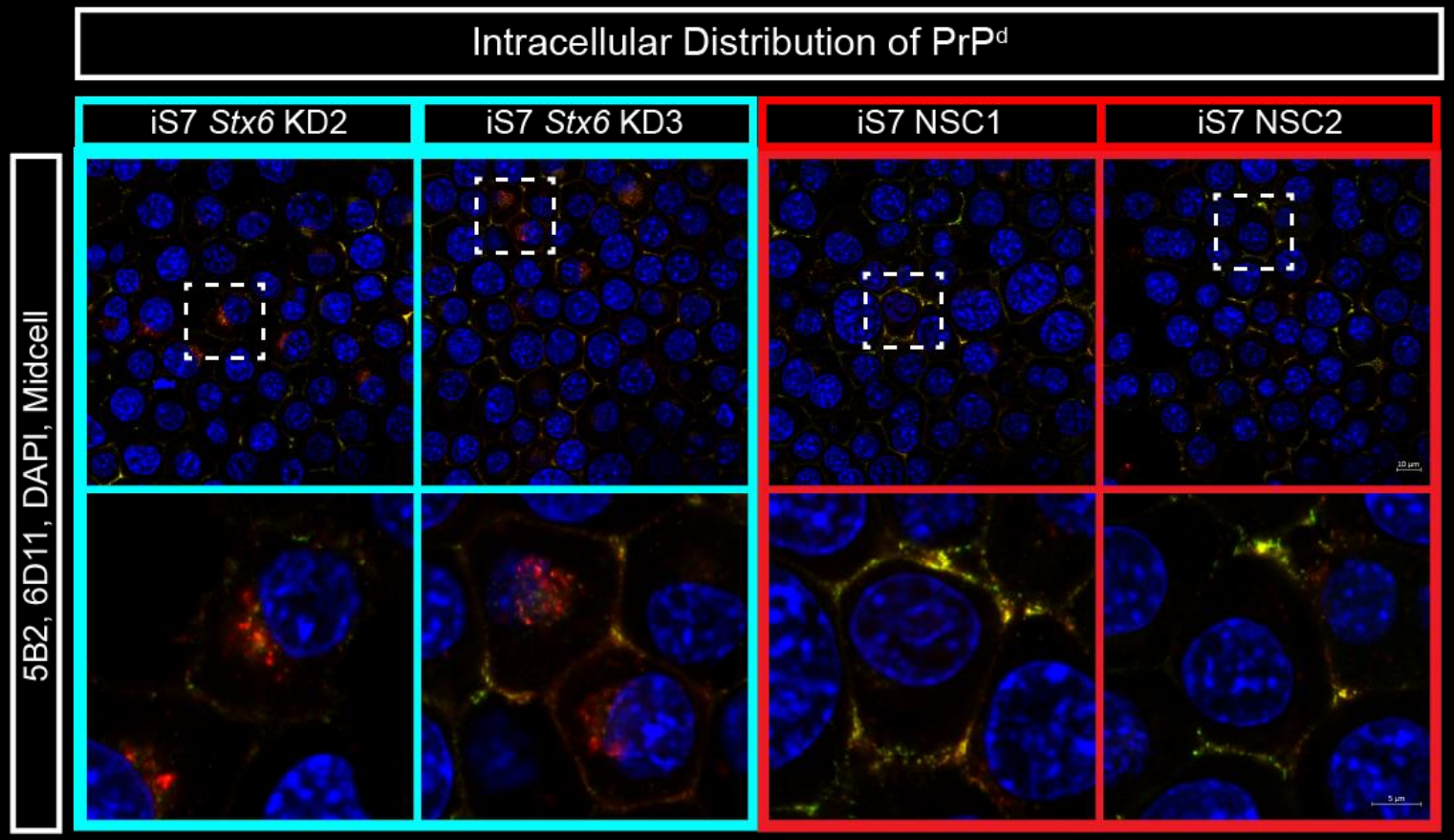
Dilution	*Stx6*$^{+/+}$ Mice		*Stx6*$^{-/-}$ Mice		Odds Ratio (95% CI)	P-Value (Genotype)
	Attack Rate	Attack Rate (%)	Attack Rate	Attack Rate (%)		
10^{-5}	13/15	86.7	11/14	78.6	1.77 (0.309-11.2)	
10^{-6}	10/13	76.9	7/13	53.8	2.86 (0.509-12.7)	**0.0500**
10^{-7}	3/12	25.0	1/14	7.14	4.33 (0.537-59.9)	
10^{-8}	1/11	9.09	0/15	0.00	Infinity (0.152-infinity)	
Combined	27/51	52.9	19/56	33.9	**2.19 (1.01-4.56)**	

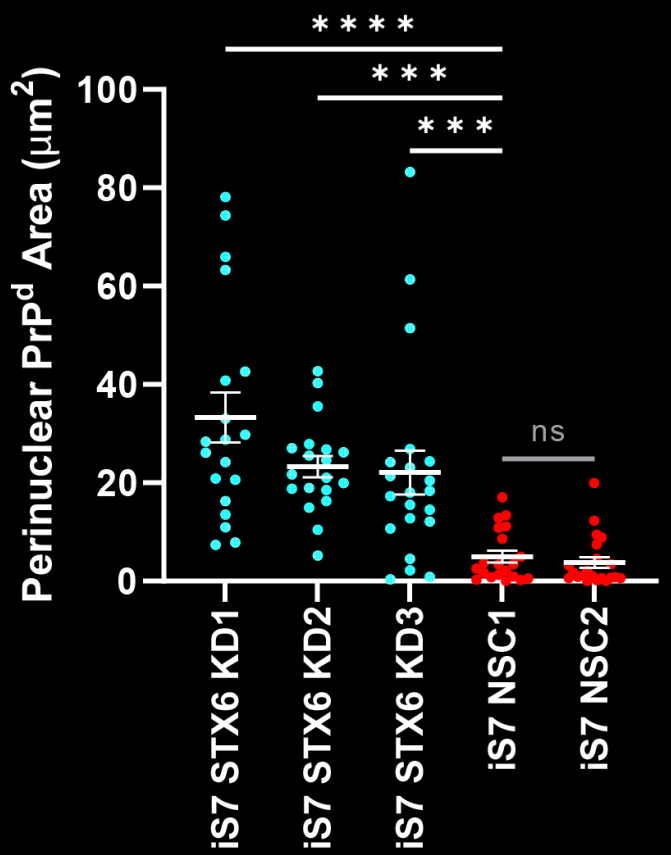
At dilutions 10^{-5} and higher, *Stx6*$^{+/+}$ mice have ~2 times higher odds of developing prion disease compared to *Stx6*$^{-/-}$ mice



Syntaxin-6 Modifies Prion-Related Phenotypes in Cellular Models with a Role in Prion Trafficking and Export

Changes in PrP^d Distribution in Chronically Infected Cells with Syntaxin-6 Knockdown





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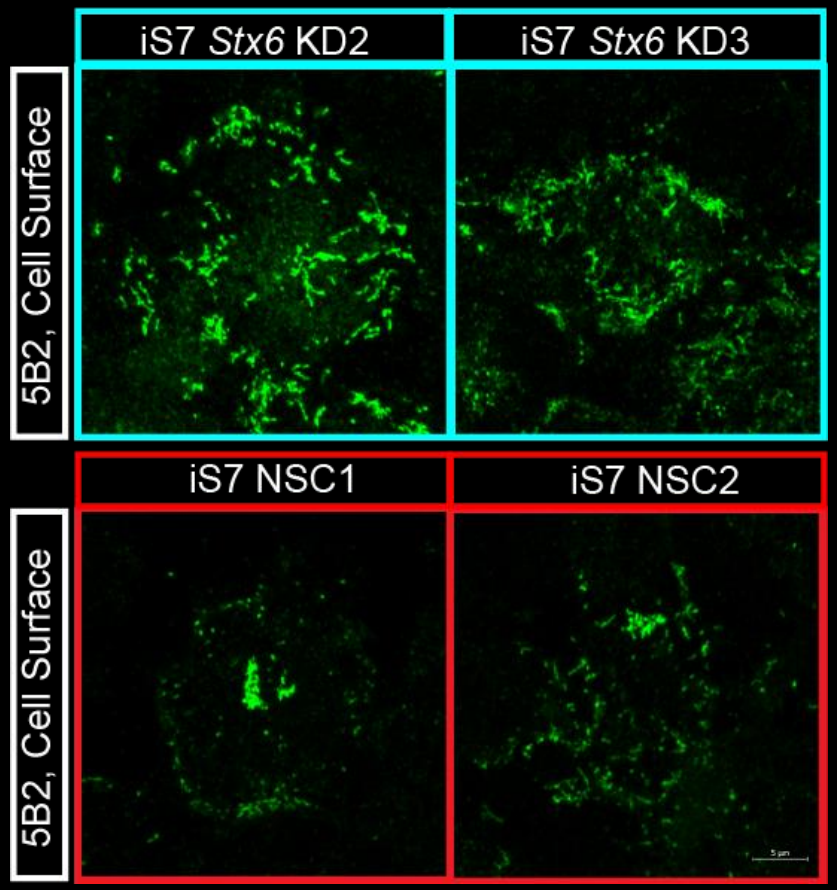
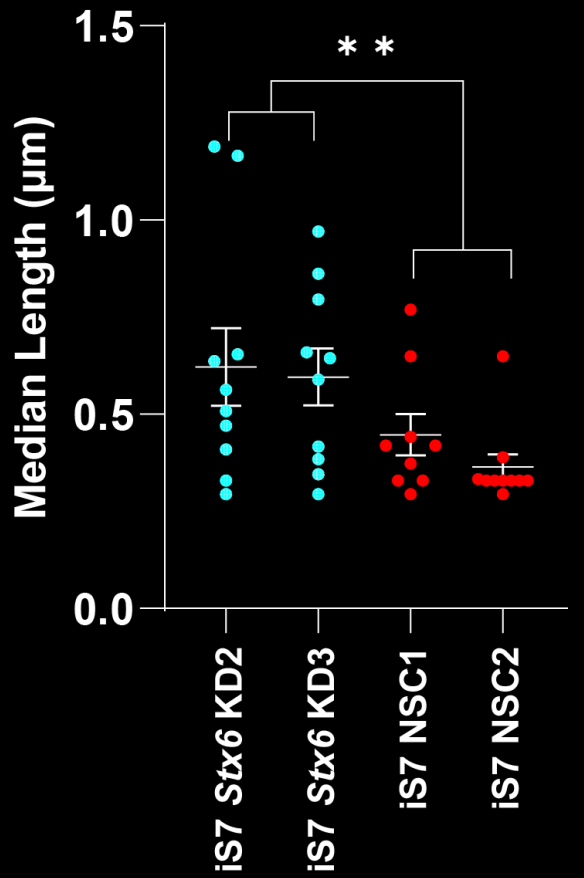
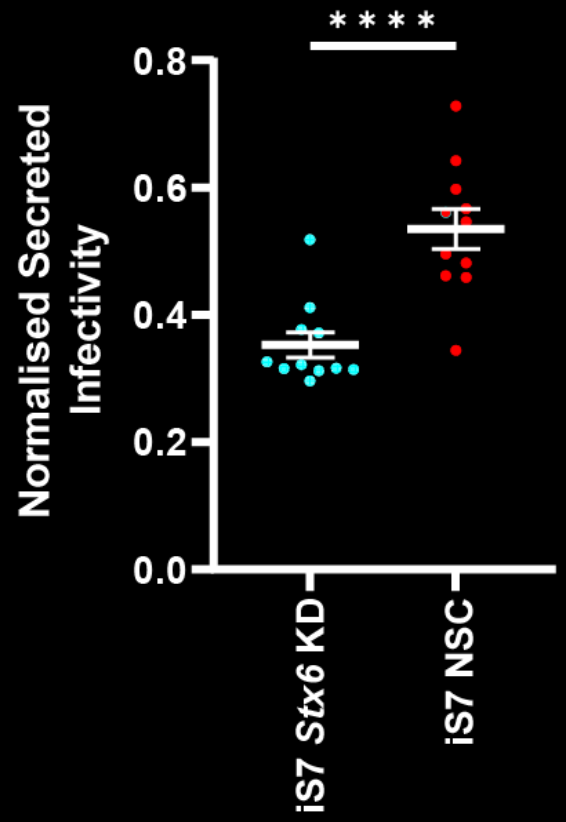




- Redistribution as opposed to increased infectivity as prion steady state levels are unchanged
- Data in keeping with an intracellular trafficking mechanism of action

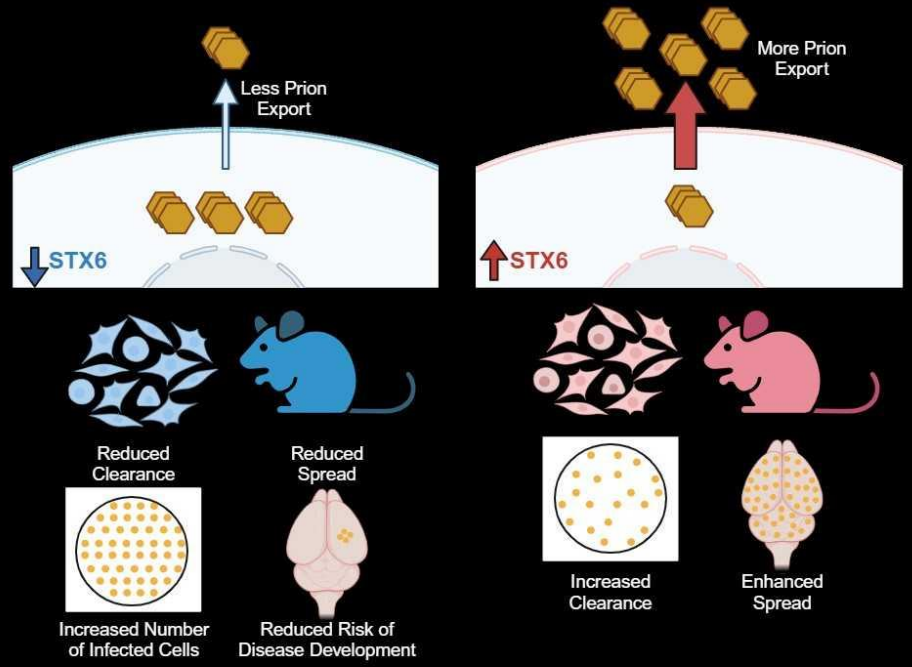
Syntaxin-6 Modifies Prion-Related Phenotypes in Cellular Models with a Role in Prion Trafficking and Export

Altered Prion Export in Chronically Infected Cells with Syntaxin-6 Knockdown



Following transient *Stx6* knockdown, conditioned media was applied to PK1 reporter cells

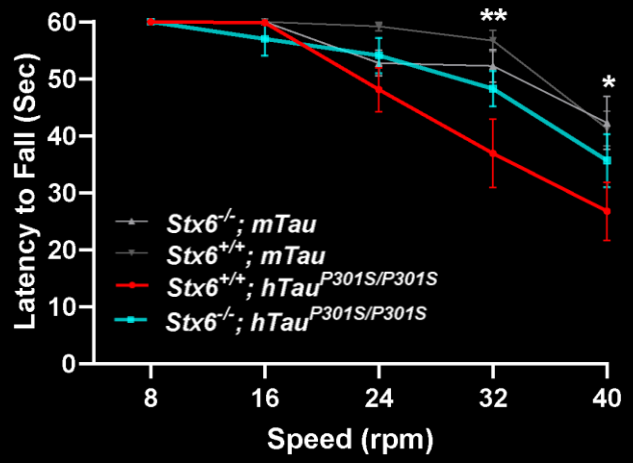
Syntaxin-6 is involved in prion export.



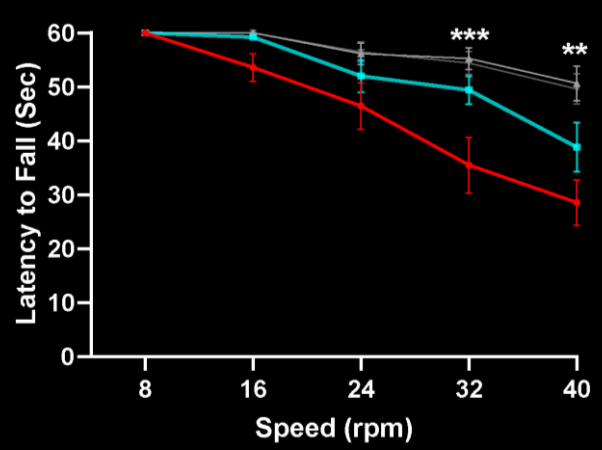
Protective Effect of Syntaxin-6 Knockout on Functional Outcome Measures in a Humanised Tauopathy Mouse Model

Rotarod Performance

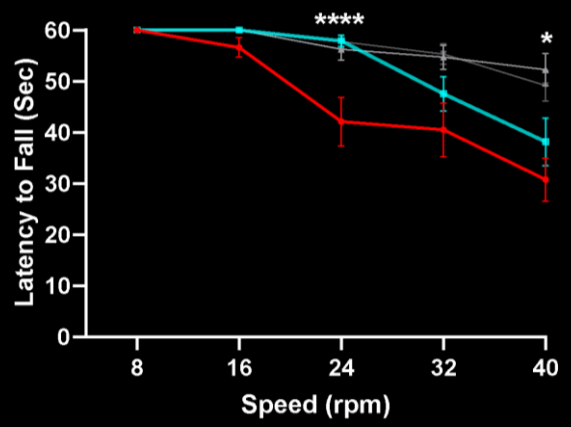
Month 1



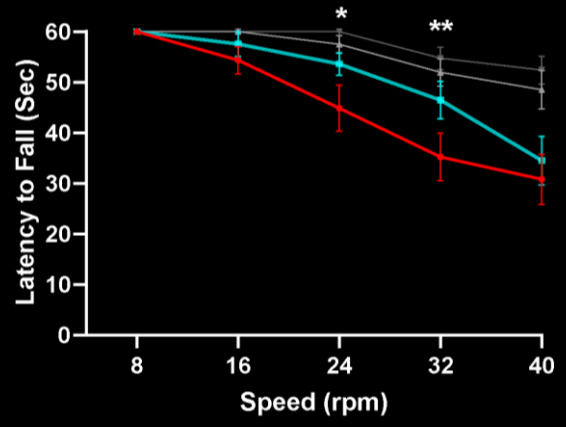
Month 2



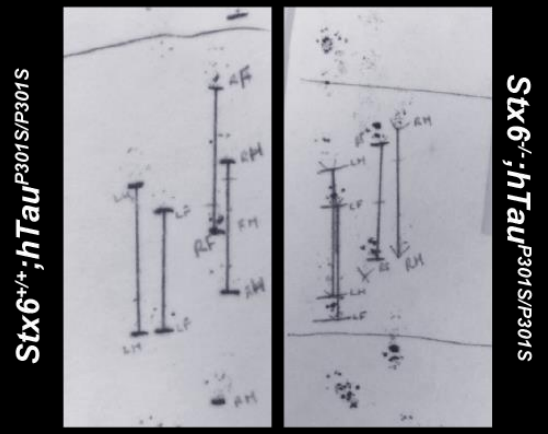
Month 3

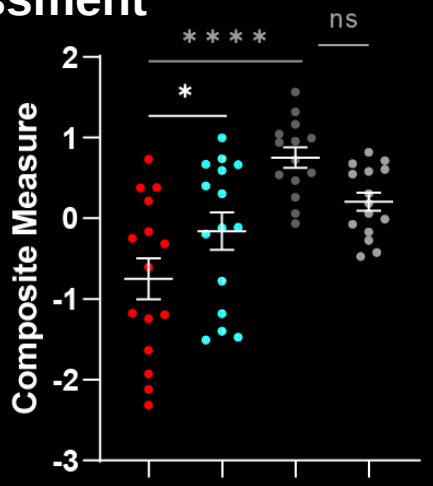


Month 4

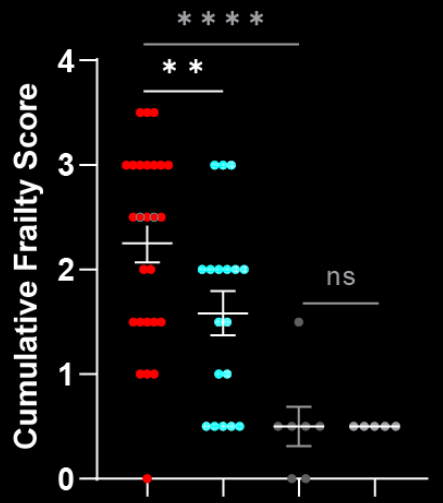


Gait Assessment





Measures of Frailty



Disease-Related Traits

1. Loss of Fur Colour
2. Coat Condition
3. Kyphosis
4. Gait Disorder
4. Tremor
6. Breathing Rate/Depth
7. Tail Stiffening

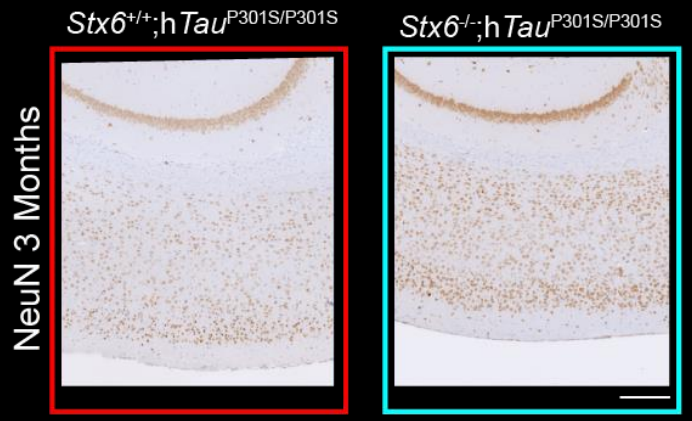
Key

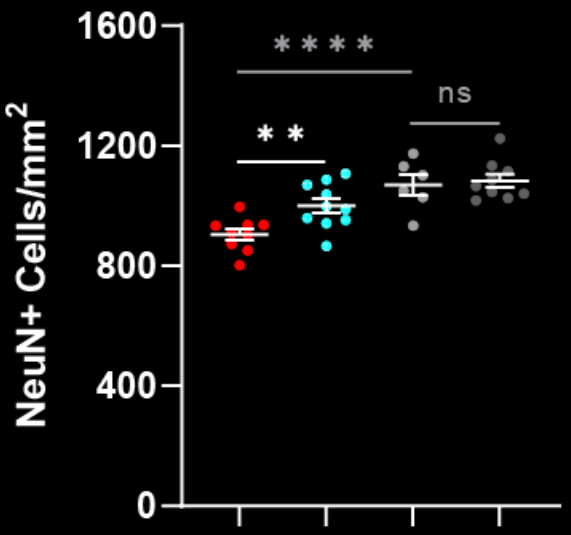
- Stx6+/+;hTau P301S/P301S
- Stx6+/+;mTau+/+
- Stx6-/-;hTau P301S/P301S
- Stx6-/-;mTau+/+

Stars refer to main experimental comparison between Stx6+/+;hTauP301S/P301S mice and Stx6-/-;hTauP301S/P301S mice.

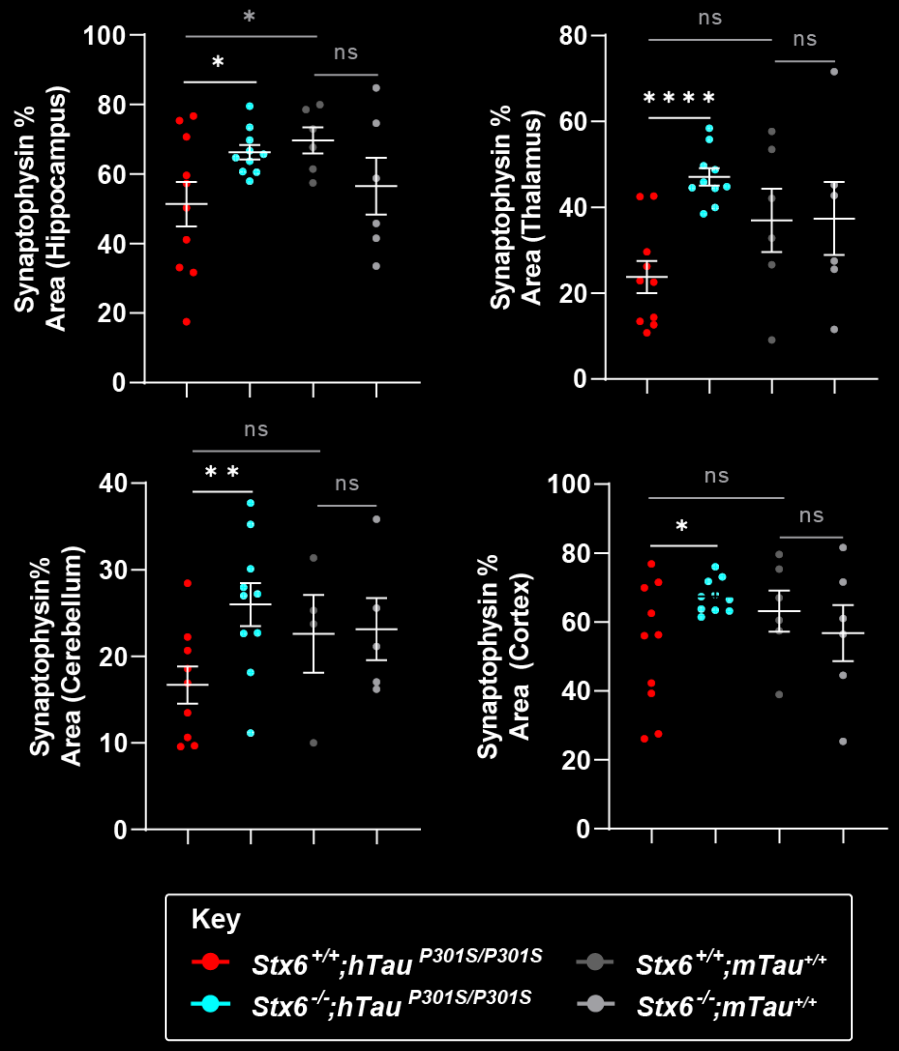
Altered Neuropathological Outcome Measures in a Humanised Tauopathy Mouse Model with Syntaxin-6 Knockout

Neuronal Loss

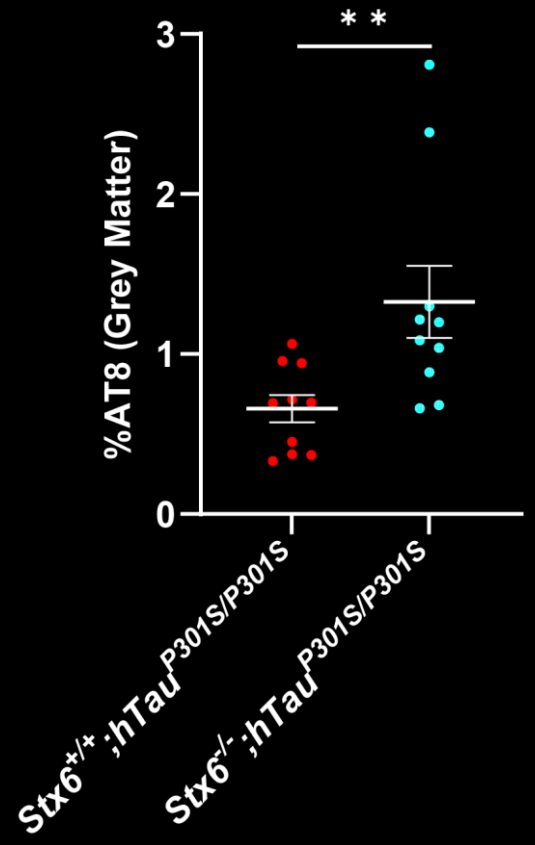




Synaptic Coverage

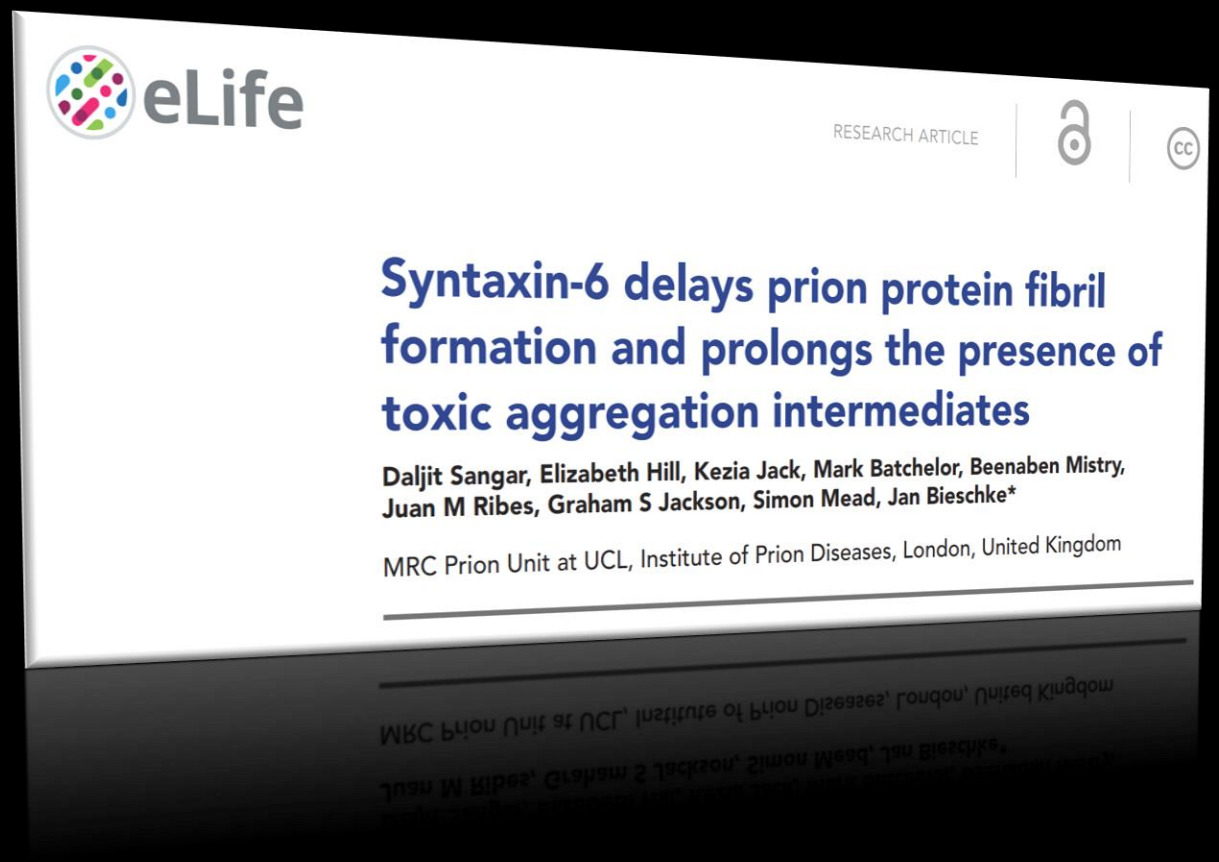


AT8-Positive Tangles



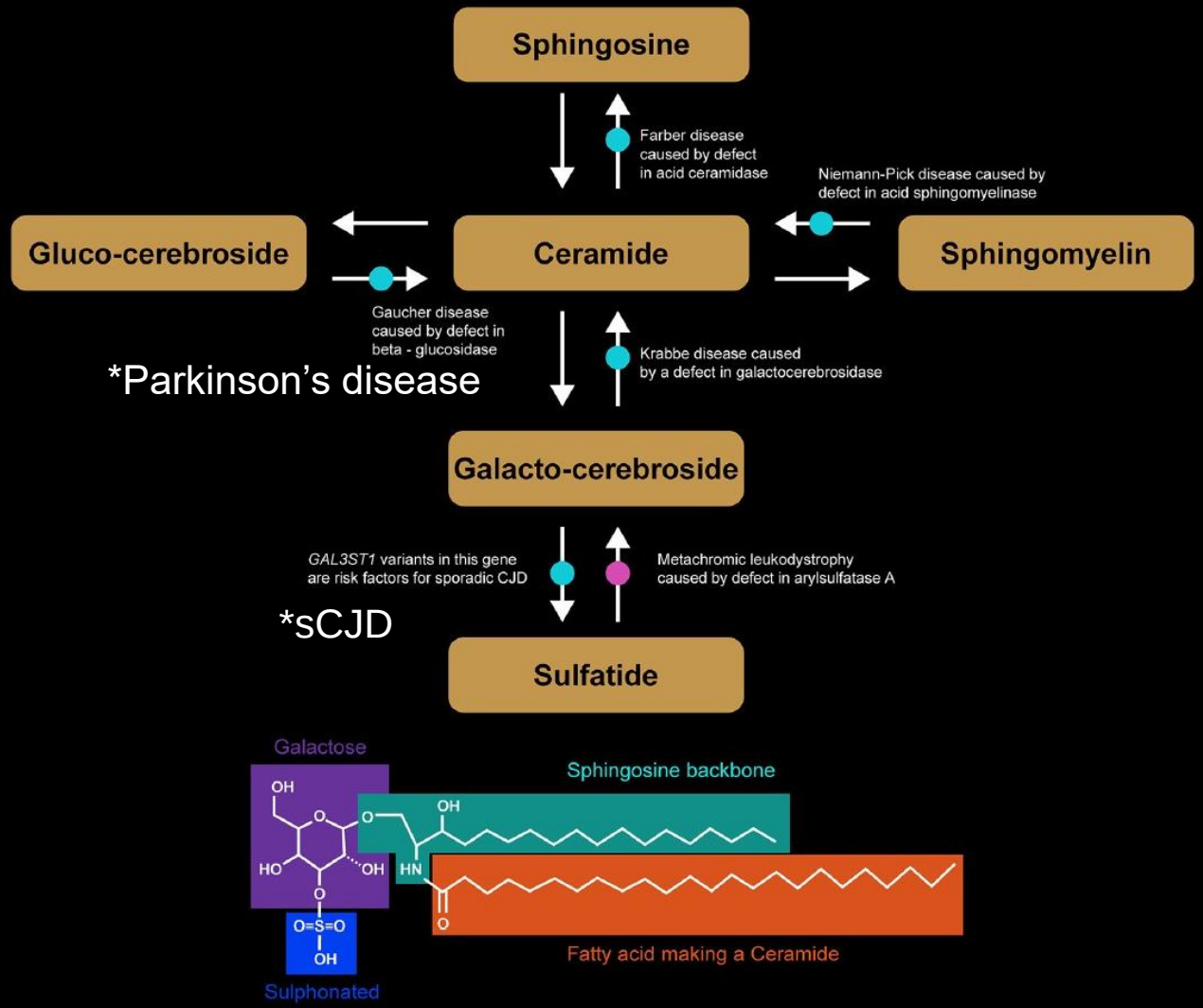
Localised increases of pathological tau species in *Stx6*$^{-/-}$;h*Tau*$^{P301S/P301S}$ mice in keeping with a role of syntaxin-6 in tau spread.

Syntaxin-6 direct interaction with prion protein fibrils



- Recombinant PrP fibril formation assay in near native conditions
- Syntaxin6 seen to bind fibrils and delayed the lag phase of their growth
- Imaging showed less ordered aggregates using EM and superresolution microscopy

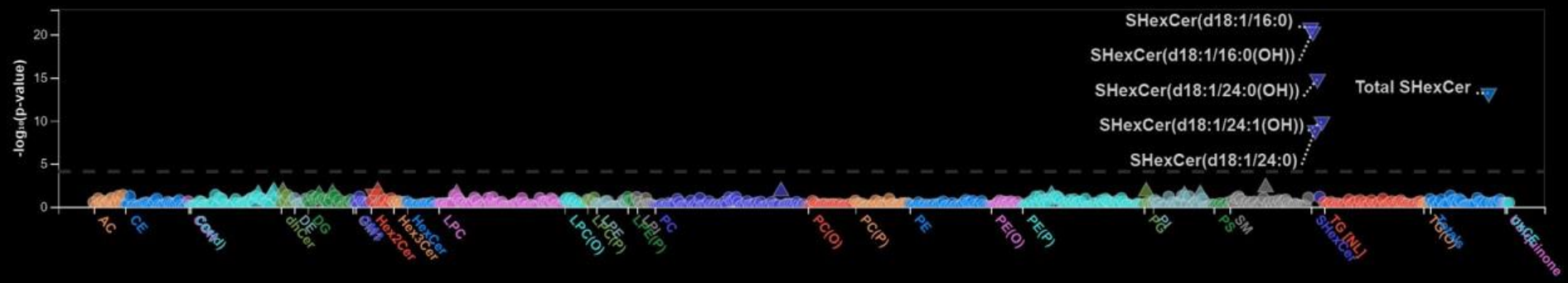
Exploring the Role of *GAL3ST1* in Prion Disease Pathogenesis



- Rs2267161-C risk factor
- A common amino acid variant (V29M) of the sole enzyme involved in the synthesis of sulfatide (*GAL3ST1* gene)
- Sulfatide = a dominant component of the myelin sheath.
- Implicates sulfatide metabolism as a novel causal mechanism in prion disease
- Pathway is littered with neurological disease genes particularly noteworthy is *GBA* in Parkinson's disease

Exploring the Role of *GAL3ST1* in Prion Disease Pathogenesis

Hypothesis of Direction of Effect



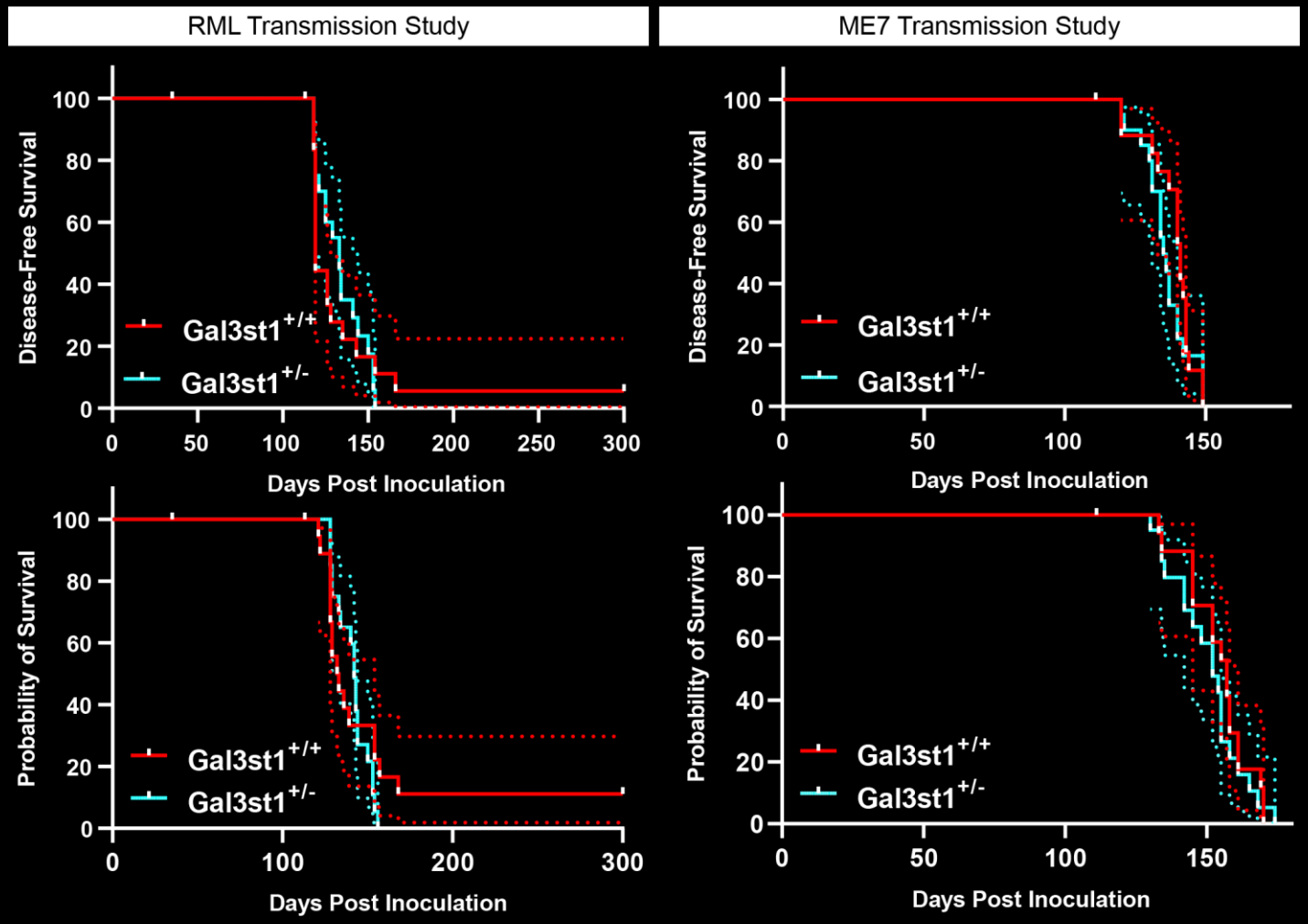
The 29V allele is associated with CJD risk and increased sulfatide levels in blood vs the 29M allele (total & five classes of sulfatides, $P=2.5x10^{-15}$ – $2.7x\ 10^{-37}$)

The V29M polymorphism is associated with 147 different brain imaging phenotypes $P<10^{-6}$ – $P<10^{-21}$ strongest association with diffusion weighted imaging suggesting change to white matter tracts in UK Biobank research imaging data

We hypothesise that certain sulfatides or premetabolites act as cofactors for prion nucleation, propagation or toxicity

Sulfatides are notable anionic components of lipid membranes and concentrate in lipid rafts with Prion protein

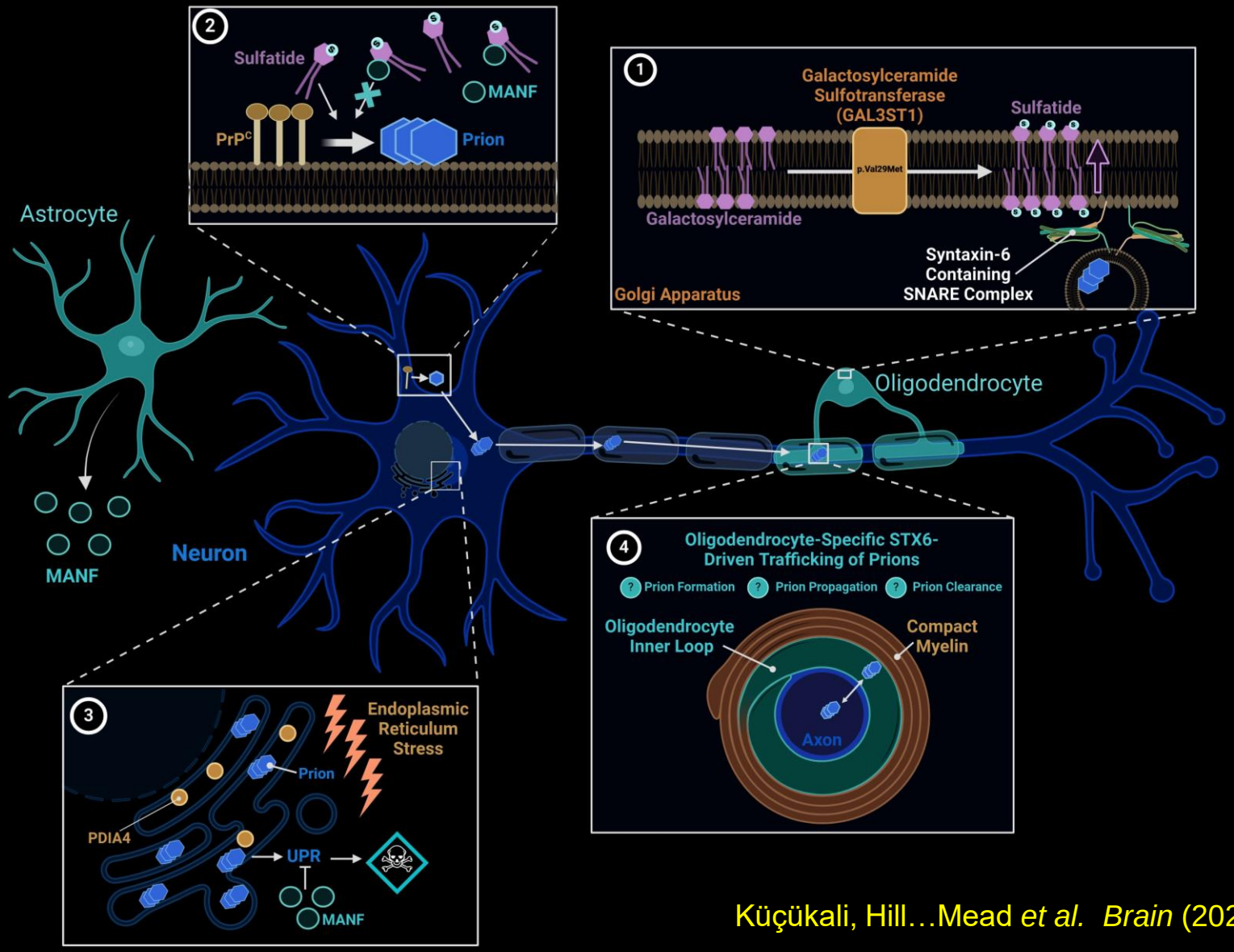
Prion Transmission Study in Mice with Knockout of Sporadic Creutzfeldt-Jakob Disease Risk Gene, *Gal3st1*



Comparable Clinical Endpoint Measures

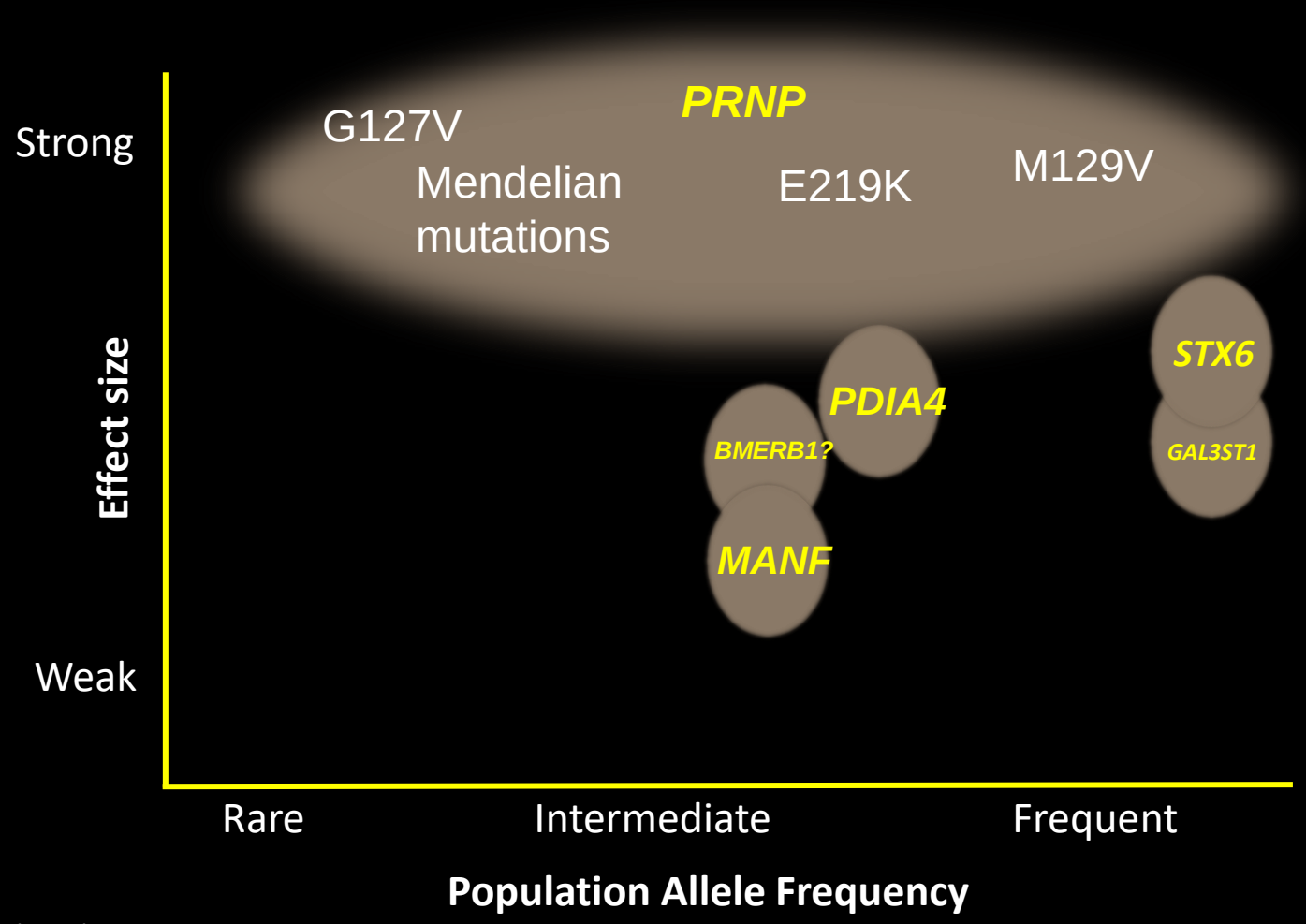
Comparable Biochemical and Neuropathological Hallmarks

Speculatively how might risk genes work in CJD?



Küçükali, Hill…Mead *et al.* *Brain* (2024) – under revision paper on BioRxiv.

Genetic architecture of human prion diseaes



- Genetic architecture has clear parallels with other dementia disorders
- Mendelian mutations and resistance factors in the gene which encodes abnormal protein aggregates
- Common modifiers in genes from shared pathways - intracellular trafficking, sulfatide metabolism

Conclusions

- No new therapeutic targets can be justified by human genetics, so far, but note mechanism of protection at *PRNP* is dominant negative not LoF
- Some possible mechanisms discussed for non-PrP risk factors which seem to be acting a very early stages of disease, not so relevant to sCJD treatment
- Role of oligodendrocytes promoted (*STX6*, *GAL3ST1*) no microglial risk gene signature in prion unlike Alzheimer's disease
- Modifiers of clinical phenotype != Risk, an area to develop
- ADRD – Overall weak correlations but some risk genes and metabolic pathways of interest are shared, presumably reflecting prion-like mechanisms
- Future work: genome sequencing, larger samples esp. in disease duration, populations outside of European ancestries, further testing in model systems

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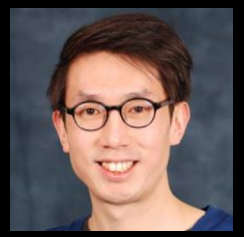
Tze How Mok


Peter Rudge


Regina Appenteng


Tom Coysh


Leah Holm-Mercer


Rachel Williams


Kirsty McNiven


Veronica O'Donnell


Rowena Baker


Jennifer Foley


Lily Farakish

National Prion Clinic

www.ucl.ac.uk/national-prion-clinic/clinic-staff

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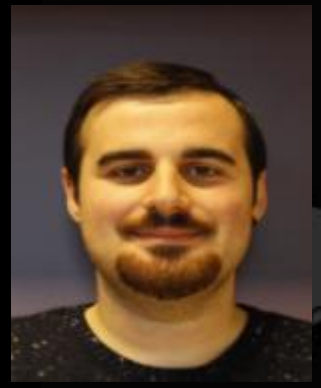
GWAS Acknowledgements



Fahri Küçükali, PhD
Postdoctoral Researcher
VIB – UAntwerp Center for Molecular Neurology
Complex Genetics of Alzheimer's Disease Group

PI Professor
University of Antwerp
VIB CMN - Genetics of Alzheimer's Disease

NCJDRSU
Richard Knight

Sweden

MRC Prion Unit at UCL
Holger Hummerich
Emma Jones
Lee Darwent
Tracy Campbell
Emmanuelle Vire
John Collinge
NPC Staff

Denmark
Ausrine Areskeviciute
Eva Lund

Netherlands
SJ van Der Lee
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Jean-Louis Laplanche
Jean-Philippe Brandel
Elodie Bouaziz-Amar

Germany
Inga Zerr
Saima Zafar
Peter Hermann

Austria
Gabor Kovacs
Ellen Gelpi
Herbert Budka

USA
Brian Appleby
Katie Glisic
Michael Geschwind
Theresa Driscoll
Kolette Cho
Jiri Safar
Pierluigi Gambetti

Spain
Miguel Calero
Pascual Sanchez Juan
Olga Calero
Antonio Salas
Raquel Sanchez-Valle

Switzerland
Herbert Budka
Adriano Aguzzi
Karl Frontzek

Italy
Piero Parchi
Fabrizio Tagliavini
Maurizio Pocchiari
Anna Poleggi
Anna Ladogana
Anna Bartoletti-Stella
Sabina Capellari
Robero Chiesa
Fabio Moda

Australia
Steve Collins
Christiane Stehmann
Shannon Sarros

New Zealand

Candidate Risk Gene Prioristization in CJD