SAPIENZA
UNIVERSITÀ DI ROMA

FATAL FAMILIAL INSOMNIA

Gene therapy to manipulate the mutated PRNP gene on chromosome 20 encoding the PrP protein with structural alterations.

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GENE THERAPY PROJECT 2022/2023 – Professors: I. Saggio, R. Burla, M. la Torre.

BACKGROUND

What is Fatal Familial Insomnia (FFI)?

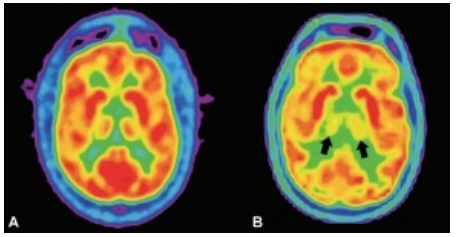


Fig. 2 Taken from L. Cracco, B. Appleby, P. Gambetti

Fatal Familial Insomnia (FFI) is an uncommon but fatal genetic disease with AD inheritance, belonging to the group of Spongiform Encephalopathies.

The median age at onset is between **50 and 60 years**.
The median survival is **16 months**.
Its prevalence is **<1 / 1 000 000 people**.

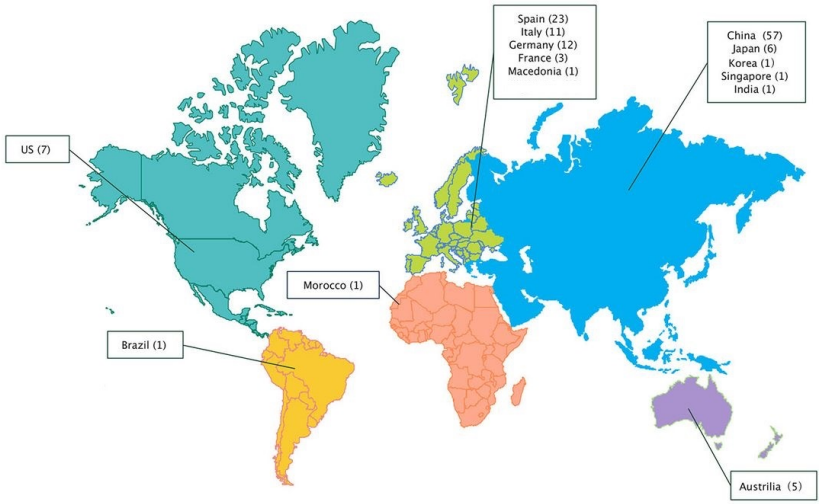


Fig .1 Adapted from Zhang J, Chu M, Tian ZC, et al. J Neurol Neurosurg Psychiatry 2022.

It's caused by a mutation in the **PRNP gene** encoding **the prion protein PrP**, that accumulating in the brain tissue, causes degeneration and death of the **Thalamic neurons.**

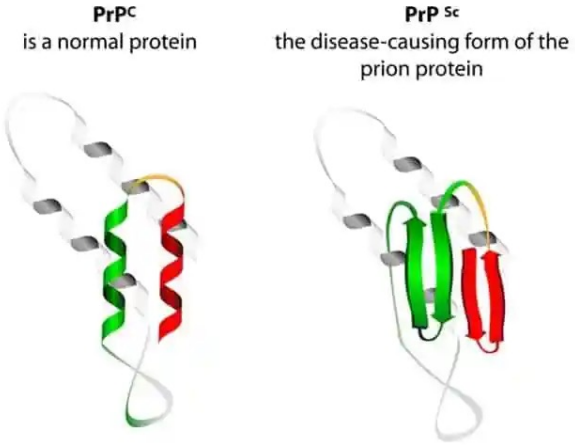


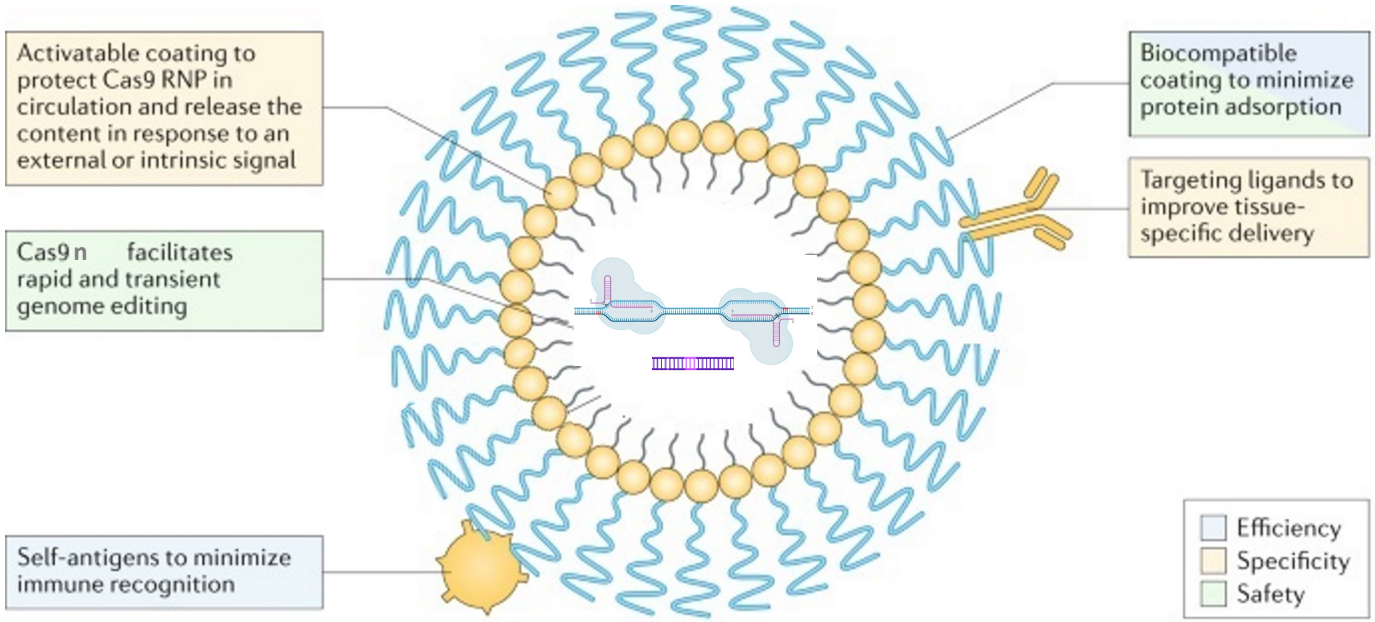
Fig. 3

Symptoms are **insomnia**, panic attacks, ataxia, hallucinations, delirium and cognitive impairment leading to death.

AIM OF THE PROJECT

To use the **CRISPR/Cas9 nickase system** to directly change the sequence of the PRNP gene in brain cells.

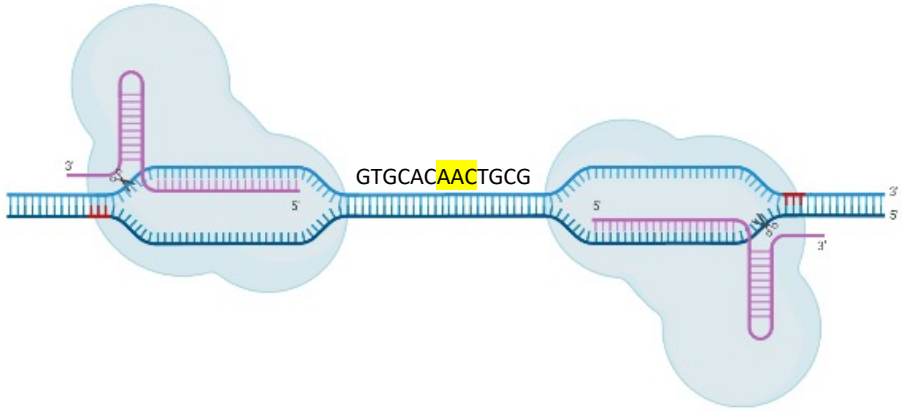
HOW?



Through **SNALP technology** to obtain **lipid nanoparticles** to carry CRISPR/Cas9 nickase system in the thalamic neurons.

Fig. 4 Modified from Tong, S., Moyo, B., Lee, C.M. et al. Nat Rev Mater 4, 726–737 (2019).

CRISPR/Cas9 nickase SYSTEM



Donor DNA temple

Fig. 5 Created on BioRender.

PRNP gene

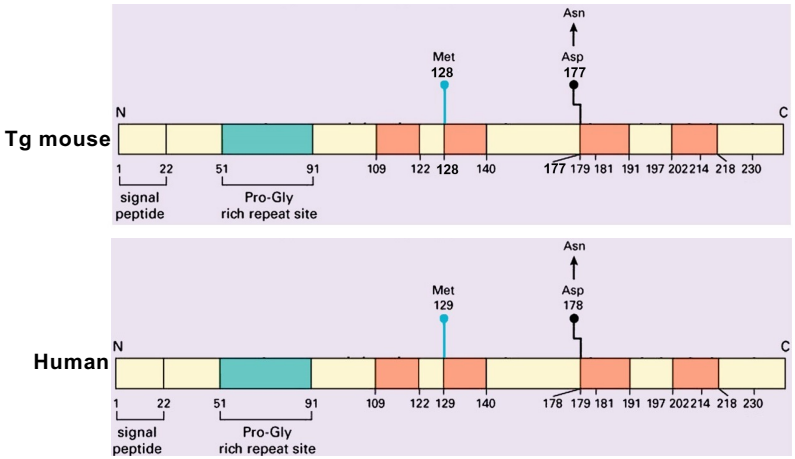


Fig. 6 Adapted from du Plessis, D. (2008). Prion protein disease and neuropathology of prion disease.

Codon 177

gRNA 1	5’ TATCACCATCAAGCAGCACA 3’
gRNA 2	5’ CTTGTTGAAGCACGTGCTGA 3’
gRNA 3	5’ CACGGTCACCACCACCACCA 3’
gRNA 4	5’ CCAGTGGATCAGTACAGCAA 3’

APPROACH

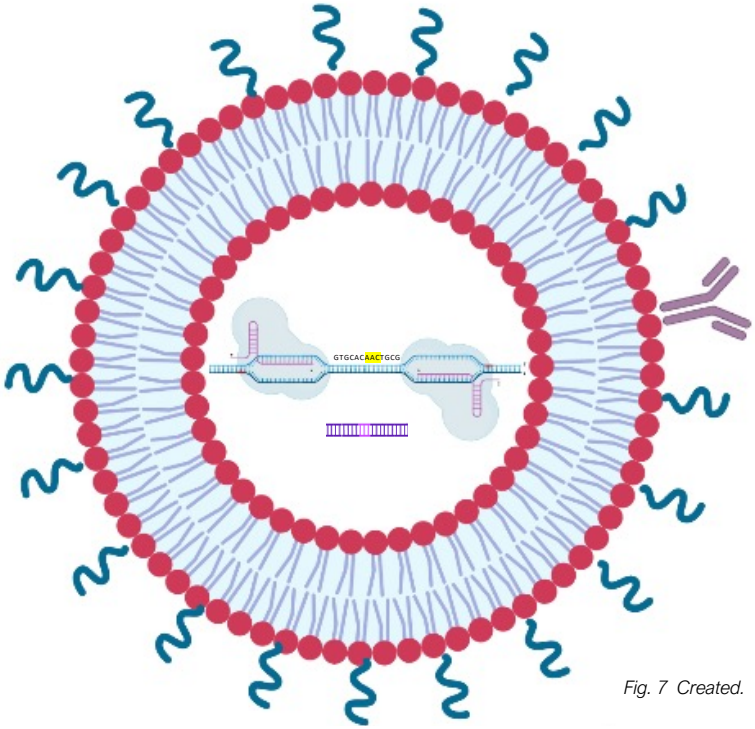
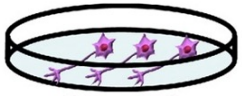


Fig. 7 Created.

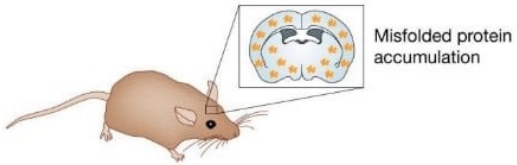
In Vitro



Neurons from
Tg(Prnp*M128*D177N)
FFI-10Rchi mice

In Vivo

Tg(Prnp*M128*D177N)FFI-10Rchi



EXPERIMENT *IN VITRO*

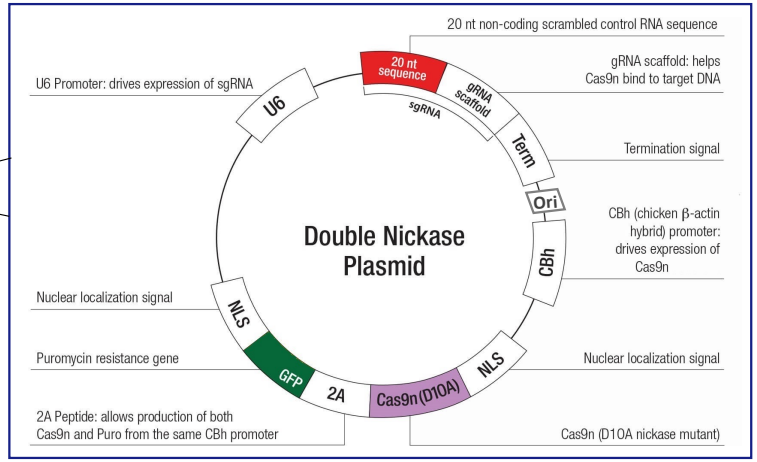
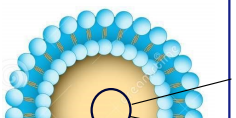
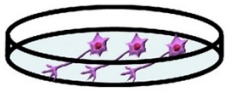
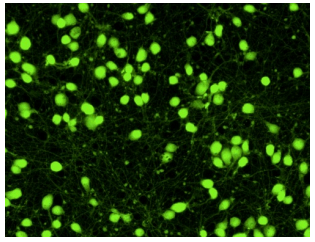


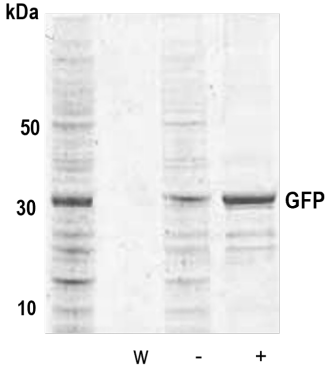
Fig. 8 *Adapted from Santa Cruz Biotechnology.*

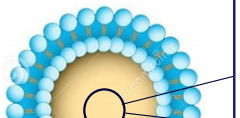




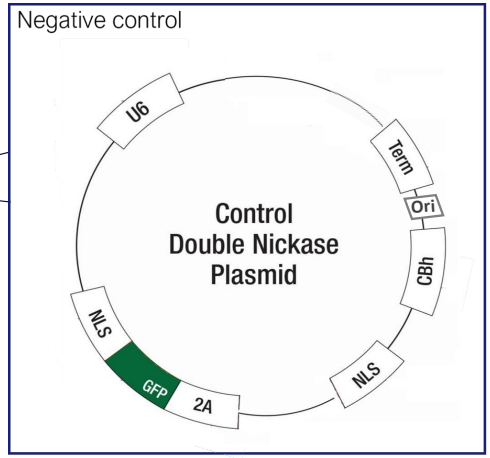
FFI neurons

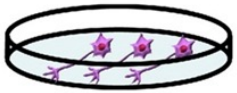




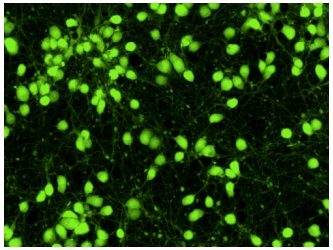


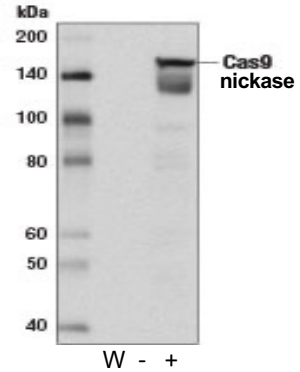
Negative control



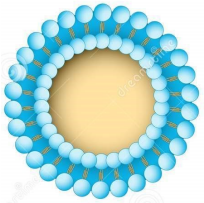


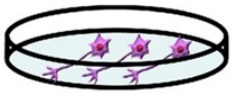
FFI neurons



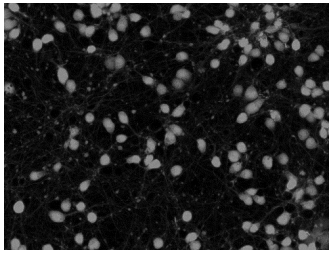


White



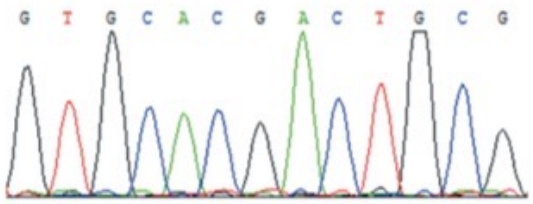


FFI neurons



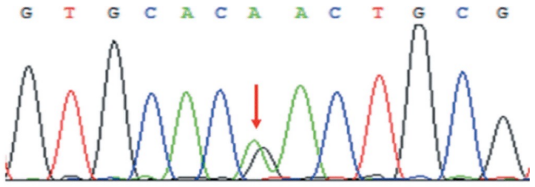
EXPECTED RESULTS *IN VITRO*

Neurons with CRISPR/Cas9n

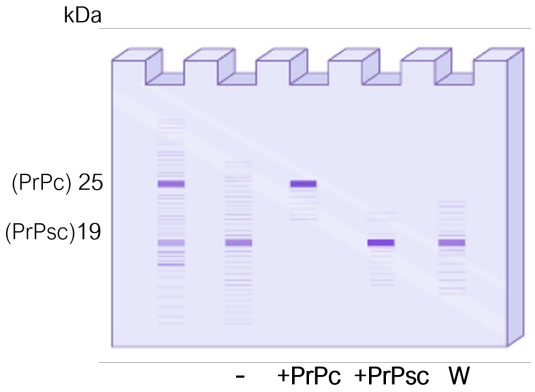


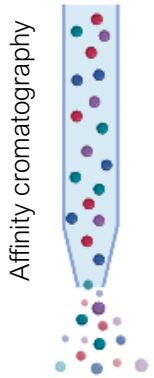
Codon 177

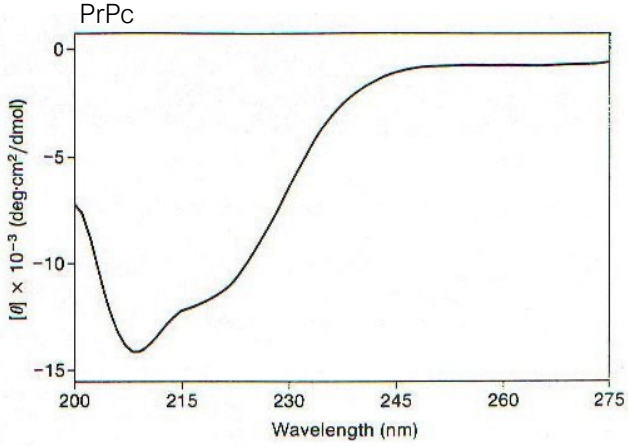
Neurons without CRISPR/Cas9n



Codon 177







Circular dichroism

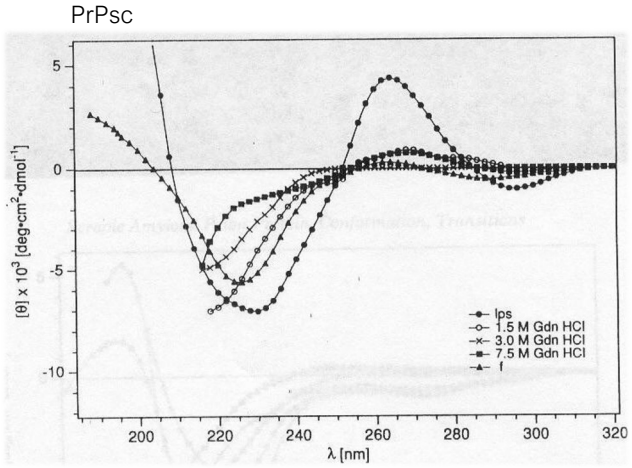
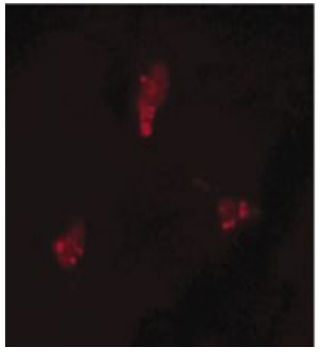


Fig. 9 Taken from https://digilander.libero.it/marcofranceschin/Prioni/prioni2b.htm

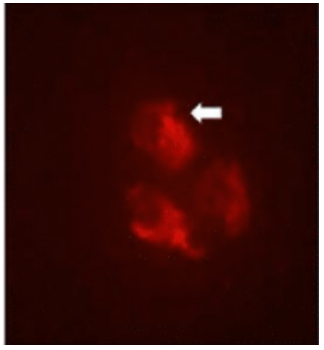
FUNCTIONAL ANALYSIS

1. FRET

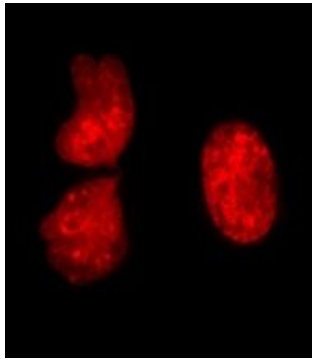
Neurons treated with CRISPR



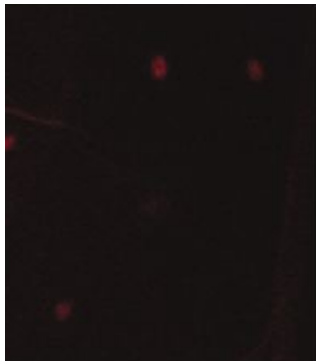
Neurons treated without CRISPR



Neurons no treated

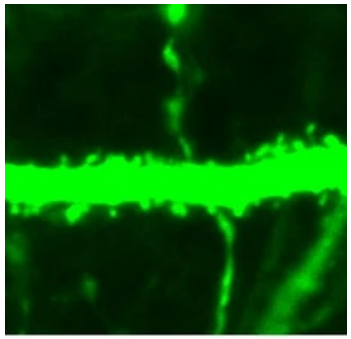


Wild type

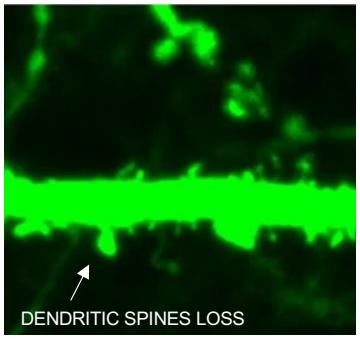


2. MORPHOLOGICAL ANALYSIS

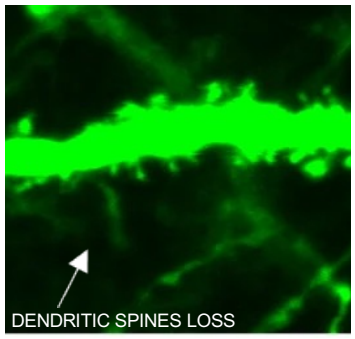
Neurons treated with CRISPR



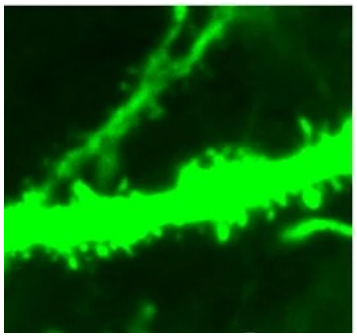
Neurons treated without CRISPR



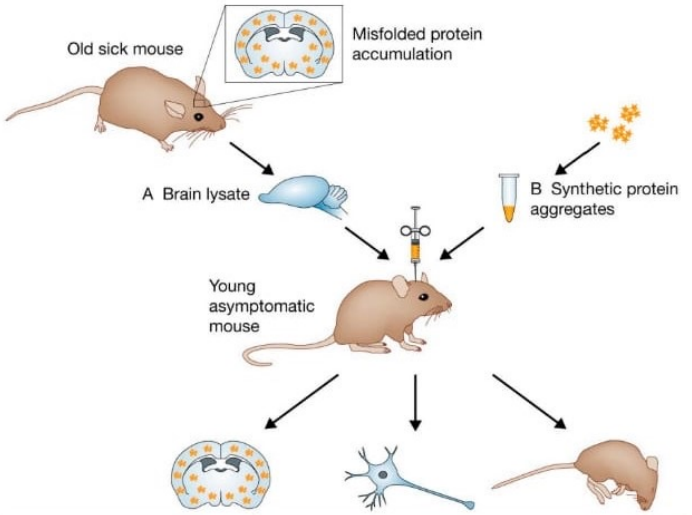
Neurons no treated



Wild type



EXPERIMENT *IN MOUSE Tg (Prnp*M128*D177N)FFI-10Rchi*



	Induction of aggregation		Neuronal loss		Fatal disease	
	Lysate	Synthetic	Lysate	Synthetic	Lysate	Synthetic
PrP^{Sc}	Yes[1]	Yes[2]	Yes[1]	Yes[2]	Yes[1]	Yes[2]

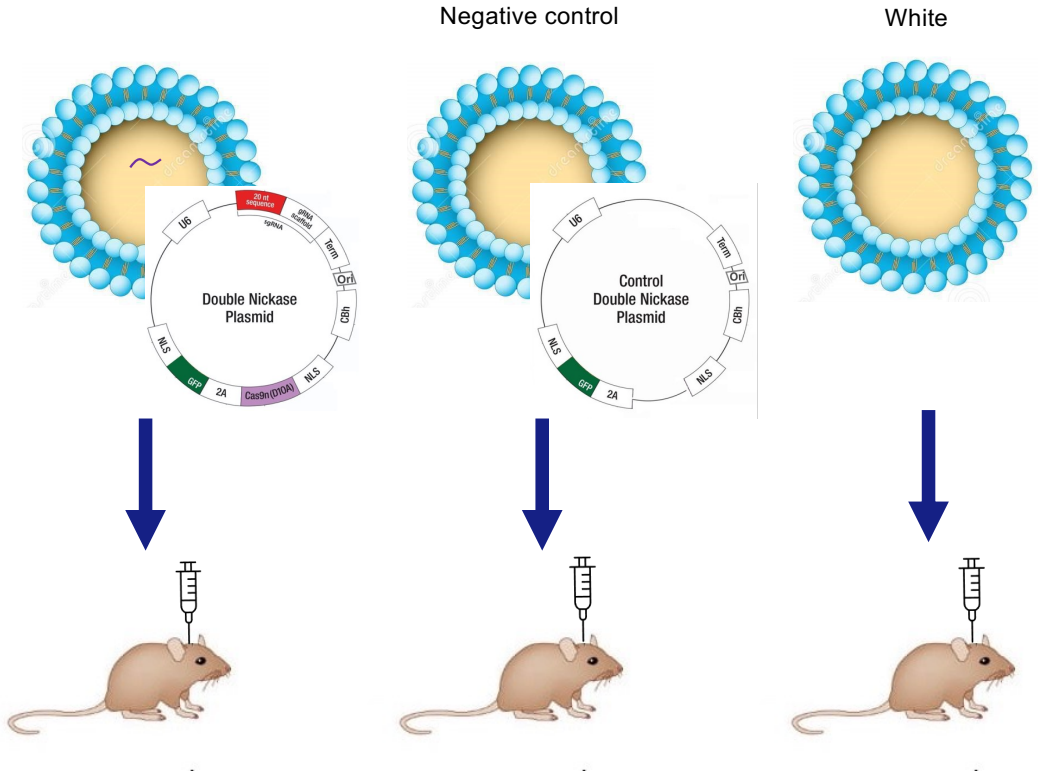
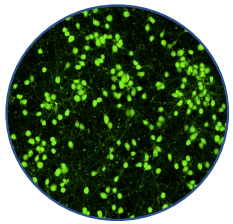
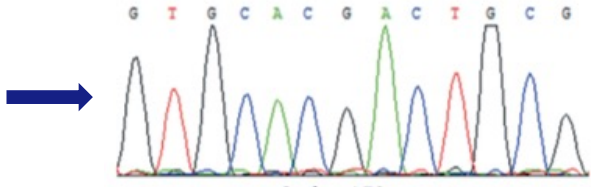


Fig. 10 *Polymenidou M, Cleveland DW. Prion-like spread of protein aggregates in neurodegeneration. J Exp Med. 2012 May.*

EXPECTED RESULTS *Tg (Prnp*M128*D177N)FFI-10Rchi*

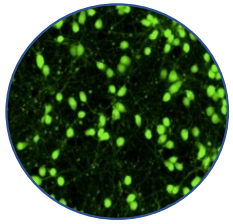
Thalamic neurons treated with CRISPR

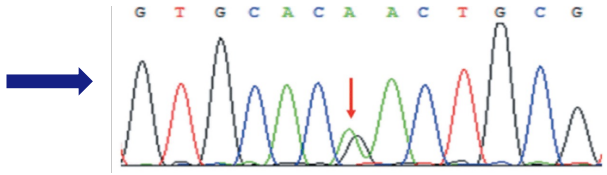




Codon 177

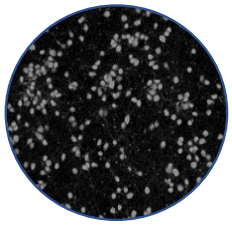
Thalamic neurons treated without CRISPR





Codon 177

Thalamic neurons no treated



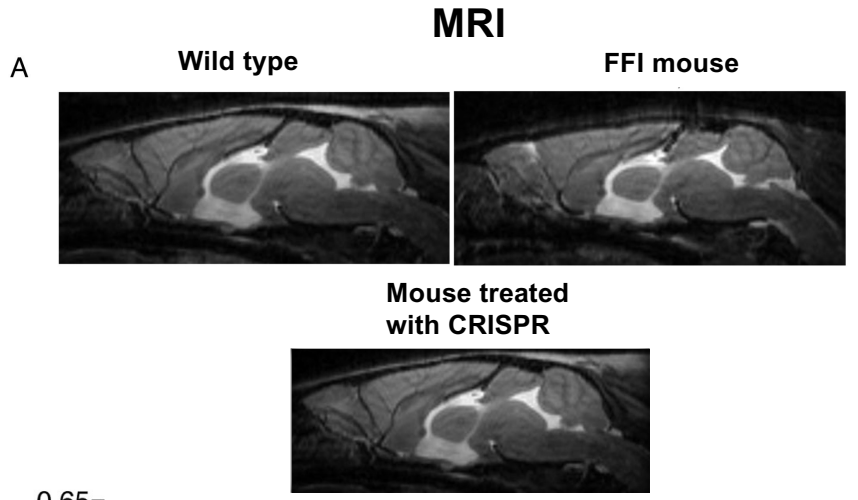
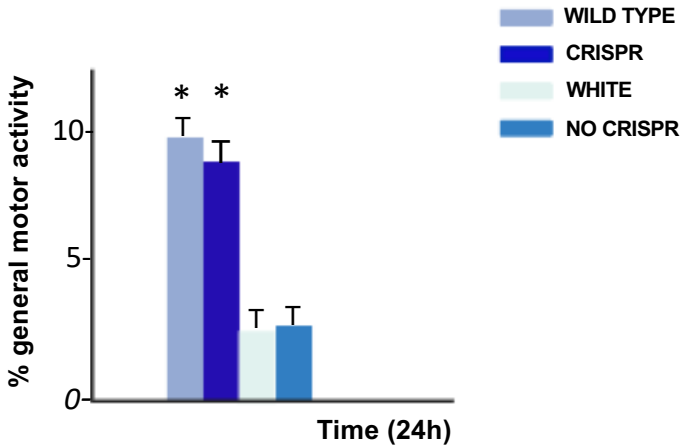
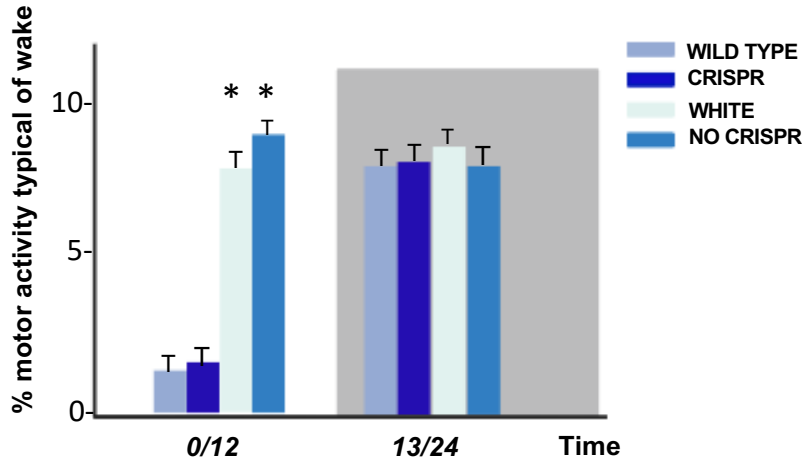


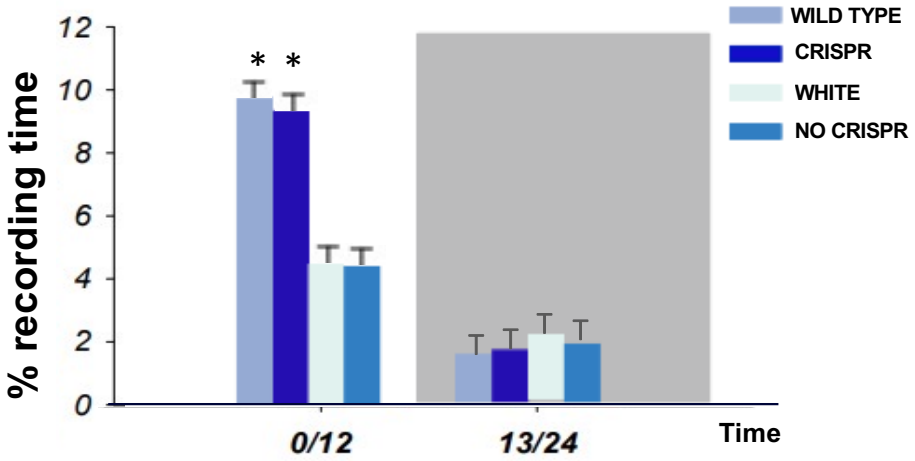
Fig. 11 Adapted from Bouybayoune I et al. (2015) Transgenic Fatal Familial Insomnia Mice Indicate Prion Infectivity-Independent Mechanisms of Pathogenesis and Phenotypic Expression of Disease.

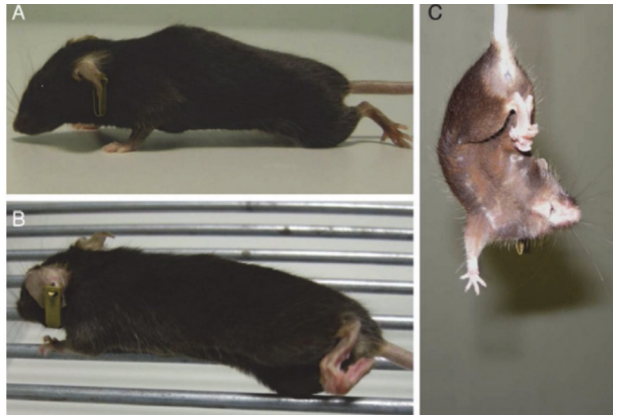
AUTOMATED MOUSE BEHAVIORAL ANALYSIS





REM SLEEP





A. Kyphotic posture
B. Paralyzed front legs
C. Foot-clasp reflex

Fig. 12 *Taken from Neurological Illness in Transgenic Mice Expressing a Prion Protein with an Insertional MutationChiesa, Roberto et al.Neuron.*

CONCLUSIONS

We demonstrated that the **liposome**-delivered **CRISPR/Cas9n system** is able to prevent the PrPSc phenotype from PrPc by repairing the genotype on the mutated codon 177.

It has been seen through behavioral analysis that the late phenotype can't be reversed, but through **preventive treatment** it is possible to block the progression of the disease.

PITFALLS

The number of **sgRNAs** sufficient to direct the CRISPR/Cas9n system to the target;

Potential **off-target effects** of CRISPR on the genome;

To increase the **efficiency** of HDR in post-mitotic cells it is necessary to inhibit the NHEJ system*.

PERSPECTIVES

More studies on human in vitro cells are needed before moving on to **human in vivo experiment.**
We hope to arrive at in vivo therapy, given the following advantages:

- Local non-viral delivery of CRISPR/Cas9n provides transient expression;
- Potential curative therapy from single dose;
- Permanent gain of function with targeted gene insertion.

Furthermore, reaching the thalamus wouldn't represent a limit for humans thanks to the use of **stereotaxic surgery**, a non-invasive procedure already used in humans to reach the deep parts of the brain with ultra-precision.

COSTS AND MATERIALS

TOT. € 389.600

WHAT?	HOW MUCH?	WHERE?
PEG	50 €	DI GIOVANNI
Lipofectamine	400 €	ThermoFisher SCIENTIFIC
Antibody scFv46.1	8900 €	ProteoGenix
Plasmid	650€/10 µg	SANTA CRUZ BIOTECHNOLOGY
Ultracruz® Transfection Reagent	177€/2 ml	ThermoFisher SCIENTIFIC
Transfection medium for Plasmid	8 €/20 ml	Roche
Western Blot kit	2000 € ca	illumina
Pirosequencing 454 Roche	1000 €	External Laboratory
BLESS- seq	500 € ca	External Laboratory
Affinity Cromatography kit	150 €	External Laboratory
Spectropolarimeter	80 €	External Laboratory
BLACK-Comet Spectrophotometer	50 €	The Jackson Laboratory
Tg mouse	800 € ca	External Laboratory
Mouse stabulation	10.000 €	External Laboratory
MRI	50 € ca	
Polygraphic analysis	300 € ca	
Research team	150.000€/year	

REFERENCES

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