Research proposal:

Permanent and reliable inactivation of Huntington's disease mutation via customized CRISPR/SaCas9 gene editing

Gal·la Vinyes i Bassols
Bachelor's degree in Biotechnology 2017-2021
UAB Universitat Autònoma de Barcelona

BACKGROUND

Huntington's disease (HD) is a severe autosomal dominant neurodegenerative disorder typically diagnosed between the ages of 40 and 60. Clinically, it is characterized by a combination of inevitably progressing motor, cognitive, and psychiatric symptoms due to loss of GABAergic medium spiny neurons (MSNs) in the striatum of the forebrain. There is no cure for HD so far, and death usually occurs 5-20 years after the first clinical signs emerge.

Most HD individuals present solely one mutated allele with an expanded CAG repeats in the *HTT* exon-1, leading to the production of the deleterious mutant huntingtin protein (mHTT). Therefore, allele-specific gene editing approaches deleting the mutated *HTT* exon-1 could become a potential therapy for HD patients.

HYPOTHESIS & OBJECTIVES

Allele-specific CRISPR/SaCas9 gene editing therapy targeting the mutated *HTT* exon-1 would decrease the formation of mHTT aggregates reducing the neuronal dysfunction and striatum atrophy.

Objectives:

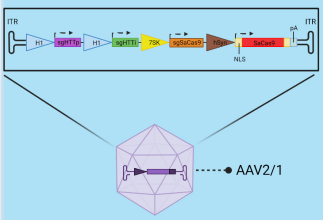
1. All-in-one vector design with neuronal tropism
2. *In vitro* reduction of mHTT aggregates in MSNs
3. *In vivo* decrease of mHTT aggregates in HD mice models
4. Therapeutic effect in HD mice models

METHODOLOGY

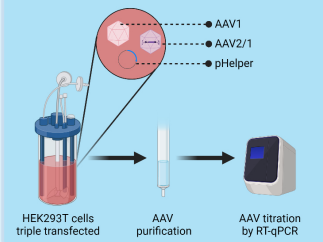
AIM 1

1.1 Construct design

By gene synthesis



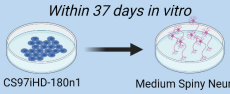
1.2 AAV production



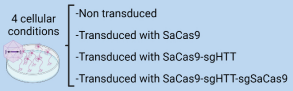
AIM 2

2.1 hiPSC differentiation into MSNs

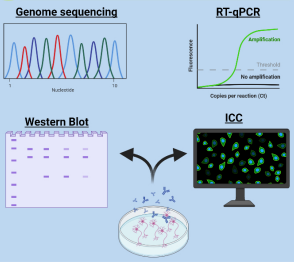
Within 37 days in vitro



2.2 Transduction of MSN



2.3 Editing analysis



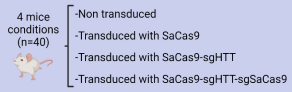
AIM 3

3.1 HD mice breeding

Hu 97/18 & Hu 128/21 mice

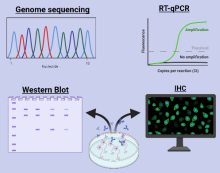
3.2 Striatal injection

Performed 4 weeks after mice birth



3.3 Editing analysis

- *Tissue extraction 8 weeks post-injection (n=20)*
- *End-stage (n=20)*



AIM 4

4.1 HD mice characterization

4.2 Therapeutic effect

**Weekly assessed from week 2 post-injection*

DISSEMINATION PLAN

Scientific dissemination:

- 2 or more publications in high-impact journals
- Results presentation in national and international congresses

Community dissemination:

- Project website creation
- Press releases and media interviews

FUTURE PERSPECTIVES

If the outcome of this research project results positive, the proposed gene therapy could be sold to pharmaceutical companies as a promising treatment for Huntington's disease either genetically diagnosed or first symptomatic individuals.

BIBLIOGRAPHY

1. Ekman, F. K., Ojala, D. S., Adil, M. M. (2019). **CRISPR-Cas9-Mediated Genome Editing Increases Lifespan and Improves Motor Deficits in a Huntington's Disease Mouse Model.** *Molecular Therapy - Nucleic Acids*, *17*, 829–839.
2. Monteys, A. M., Ebanks, S. A., Keiser, M. S. (2017). **CRISPR/Cas9 Editing of the Mutant Huntingtin Allele In Vitro and In Vivo**. *Molecular Therapy*, *25*(1), 12–23.
3. Merienne, N., Vachey, G., Déglon, N. (2017). **The Self-Inactivating KamiCas9 System for the Editing of CNS Disease Genes.** *Cell Reports*, *20*(12), 2980–2991.
4. Shin, J. W., Kim, K. H., Chao, M. J. (2016). **Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9**. *Human Molecular Genetics*, *25*(20), 4566–4576.