From Lysosomal Storage Disease iPSCs models to gene editing therapy: future perspectives

Diogo Ribeiro [1,2,3], Ana Joana Duarte [1,2,3], Olga Amaral [1,3]

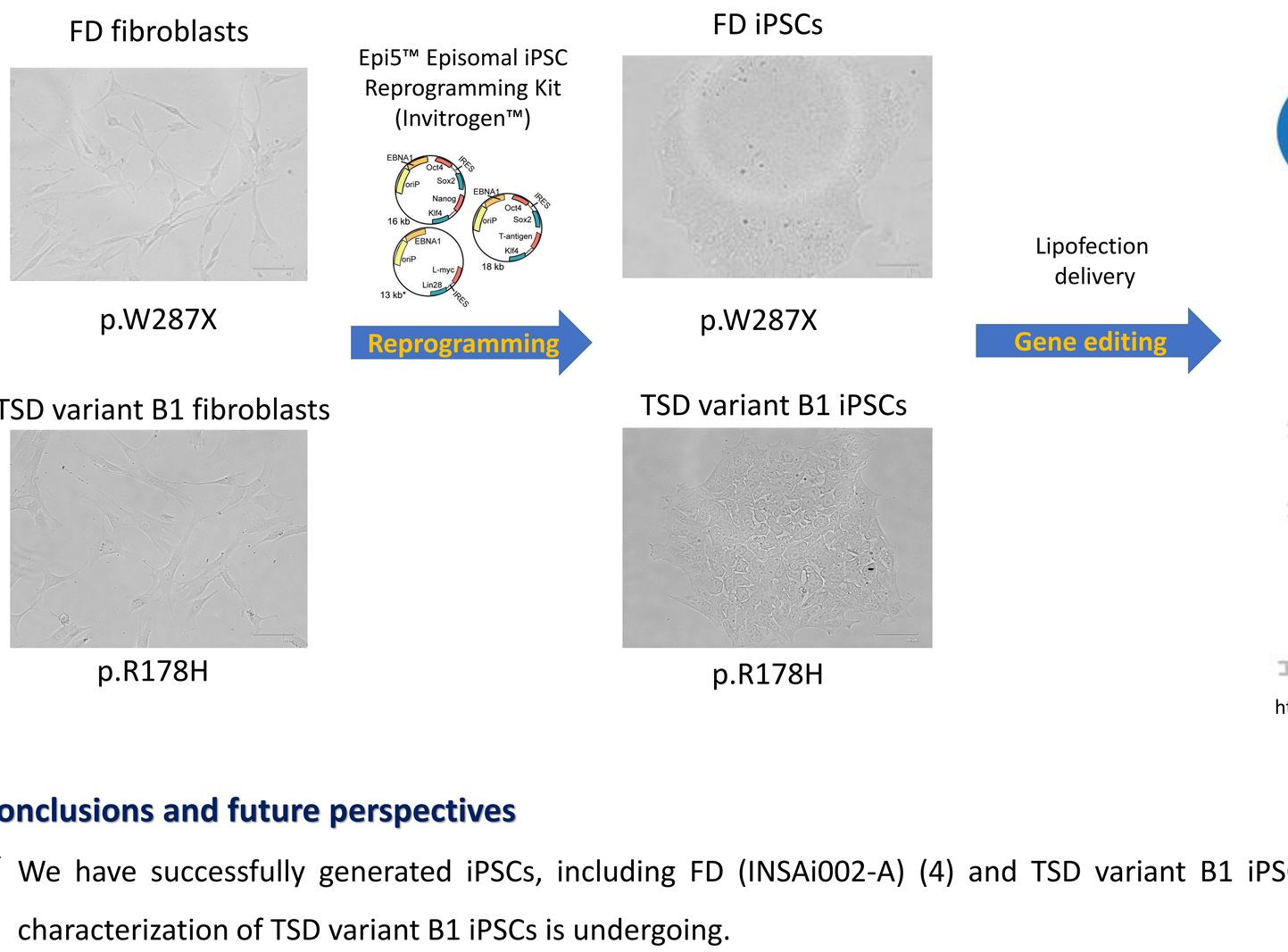
Introduction

- Lysosomal storage diseases (LSDs) are monogenic disorders characterized by accumulation of macromolecules in the late endocytic system. Its collective frequency of 1/5000 live births and are caused by inherited defects in genes that mainly encode lysosomal proteins (1). One of the most common LSDs is Fabry Disease (FD, MIM#301500).
- In the Portuguese population, lysosomal storage disorders (LSDs) have a prevalence of 1/4000 live births. Tay Sachs disease (TSD, MIM #272800) variant B1 is one of the most prevalent in the Portuguese population (2).
- FD is caused by mutations on the *GLA* gene (MIM #300644), like the mutation p.W287X (rs104894839), leading to alpha-galactosidase A impairment.
- The TSD variant B1 is caused by mutations on the *HEXA* gene (MIM#606869.0006), leading to hexosaminidase A misfunction. The mutation here studied, p.R178H (rs28941770), is frequent among population groups of Iberian heritage. This mutation has a carrier frequency of 1:340 in the Portuguese population and 1:119 in the North of Portugal (2).

Aim

In our group, we are attempting to use gene editing through CRISPR/Cas9 (3) as a therapeutic tool to correct the p.W287X FD mutation and the p.R178H TSD variant B1 mutation. Our aim is first to obtain induced pluripotent stem cells (iPSCs) derived from these two cell lines and subsequently correct the mutational defect.

Methods and Results



https://www.addgene.org/guides/crispr/

Conclusions and future perspectives

- ✓ We have successfully generated iPSCs, including FD (INSAi002-A) (4) and TSD variant B1 iPSCs. The biochemical and genetic characterization of TSD variant B1 iPSCs is undergoing.
- ✓ To perform the CRISPR/Cas9 correction we will design our single-guide RNAs (sgRNAs) on available platforms, such as Benchling (https://www.benchling.com/). This type of platforms enable the selection of the best sgRNAs: with better in-targets and lower off-targets.
- ✓ The efficacy of transfection will be evaluated using adequate software, such as TIDE (http://shinyapps.datacurators.nl/tide/). Cells with the desired correction will be selected by isolating and growing single colonies. Sequencing, cytotoxicity assays, and Next Generation Sequencing (NGS) will be used to determine the specificity of CRISPR/Cas9 editing.
- ✓ Gene editing in monogenic diseases may prove to be an effective therapeutic approach in the near future.

References

(1) Platt, F.M. (2014) Sphingolipid lysosomal storage disorders. Nature 510(7503):68-75; (2) Duarte, A.J., Ribeiro, D., Oliveira, P. and Amaral, O. (2017) "Mutation Frequency of Three Neurodegenerative Lysosomal Storage Diseases: From Screening to Treatment?". Archives of Medical Research 48 3 : 263-269; (3) Kim, E.J., Kang, K.H. and Ju, J.H. (2017) CRISPR-Cas9: a promising tool for gene editing on induced pluripotent stem cells. Korean J. Intern. Med 32, 42-61; (4) Duarte, A.J., Ribeiro, D., Santos, R., Moreira, L., Bragança, J. and Amaral, O. (2020) "Induced pluripotent stem cell line (INSAi002-A) from a Fabry Disease patient hemizygote for the rare p.W287X mutation". Stem Cell Research 45: 101794.

Affiliation and Funding

[1] Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), Departamento de Genética Humana, Unidade de Investigação e Desenvolvimento, Porto.

[2] Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Porto.

[3] Centro de Estudos de Ciência Animal (CECA), ICETA, Universidade do Porto, Porto.

Funded by the Fundação para a Ciência e Tecnologia: (FCT) Project Grant: PTDC/BIM-MEC/4762/2014 (2016)

Acknowledgements

The authors thanks the collaboration of INSA's colleagues from DGH and funding from Portuguese Foundation of Science and Technology (FCT) project PTDC/BIM-MEC/4762/2014 (PI: O.A.) and UIDB/00211/2020.

Diogo Ribeiro and Ana Joana Duarte are PhD students of Biomedical Sciences Doctoral Program of ICBAS.