**PROJECT**

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| **Supervisor:** | __Gaia Colasante_____ |
| Title: | Treating Dravet syndrome caused by dominant negative mutations |
| *Curriculum*: | _____Gene and cell Therapy_____ |
| Link to the personal page of the University or relevant hospital site website: | https://research.hsr.it/en/divisions/neuroscience/stem-cells-and-neurogenesis/gaia-colasante.html<br>https://www.unisr.it/en/offerta-formativa/medicina-chirurgia/post-lauream/dottorato-medicina-molecolare/director-of-studies/colasante-gaia |

**Description of the Project (max 3,000 characters including spaces)**

**<u>Background/gap of knowledge</u>**

Dravet syndrome (DS) is a severe developmental and epileptic encephalopathy arising in the first year of life of otherwise normal babies, characterized by refractory seizures and behavioral alterations, including autistic trait, intellectual disability and cognitive impairment. It is caused by loss- of-function (LoF) mutations in one allele of *SCN1A* gene, determining haploinsufficiency of *the* encoded alpha subunit of the voltage-gated sodium channel Nav1.1[1,2]. Gene therapy strategies aiming to rescue the haploinsufficiency of Scn1a gene by boosting the expression of the wild-type allele at both transcriptional and post-transcriptional of the gene are being developed[3–9]. We have recently identified a new mechanism controlling *SCN1A* gene expression at translational level, highlighting the presence of regulative elements in 5'untranslated region of all *SCN* genes, and showed that disruption of this regulation can enhance gene expression and rescue phenotypic defects in a DS mouse model**.** However, this approach cannot discriminate between the wild-type and mutant alleles and therefore, it enhances expression of both. While this is not an issue for classic loss-of-function (LoF) mutations, it is not suitable for dominant-negative (DN) mutations. This is because concomitant enhancement of a DN channel may adversely affect the function of the healthy copy, thereby abolishing or reducing the therapeutic effect.

**<u>Rationale and hypothesis</u>**

To address this issue and enable the development of a gene therapy suitable for patients with DN mutations, we hypothesize that enhancing the expression of a *SCN1A* paralog could

compensate for its function. In this way, we aim to restore Nav channel function without the risks associated with increasing the expression of a DN-mutant allele.

**Objectives and specific aims**

The aim of this PhD project is to develop a base editing strategy that interferes with a regulatory mechanism common to different SCN genes in order to restore the physiological function of Nav channels and compensate for Nav1.1 loss.

The project will be structured around the following aims:

1) Development of a base editing strategy to enhance the expression of Nav alpha subunits different from Nav1.1 by disrupting a previously identified regulatory mechanism in the 5′ UTR of these genes that modulates mRNA translation efficiency.

2) In vitro testing of the different strategies in primary neurons derived from a DS mouse model, with the goal of identifying the Nav subunit best able to compensate for Nav1.1 function.

3) Optimization and validation of the selected strategy in human and mouse models of DS, also carrying DN mutations in *SCN1A* gene.

**Expected outcomes**

The successful completion of this project will determine the efficacy of a novel gene therapy strategy for treating Dravet syndrome, allowing to avoid the risks associated with enhancing the mutant allele. This is particularly important in the case of DN mutations. Among the different *SCN* paralogs, we expect to identify the Nav α-subunit that, when upregulated, can most effectively compensate for *SCN1A* expression and restore proper neuronal function in Dravet background.

**Skills that the student should acquire** (max. 600 characters including spaces):

The PhD candidate will be trained to perform cutting edge molecular biology techniques and to design base editing strategy and to deliver it in relevant murine and human models of Dravet syndrome. He/she will learn to differentiate human induced pluripotent stem cells (hiPSCs) in neurons and to manipulate murine animal models. Moreover, he/she will learn to design and perform experiments related to the phenotypic assessment of Dravet mice. In more detail, to perform surgery for implant of EEG-transmitters and to record and analyze EEG activity and behavioral tests.

**References** (max. 15)

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5. Yamagata T, Raveau M, Kobayashi K, et al. CRISPR/dCas9-based Scn1a gene activation in inhibitory neurons ameliorates epileptic and behavioral phenotypes of Dravet syndrome model mice. Neurobiol Dis. 2020;141. doi:10.1016/j.nbd.2020.104954

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7. Han Z, Chen C, Christiansen A, et al. Antisense oligonucleotides increase Scn1a expression and reduce seizures and SUDEP incidence in a mouse model of Dravet syndrome. Sci Transl Med. 2020;12(558). doi:10.1126/SCITRANSLMED.AAZ6100

8. Tanhenaus A., et al. Cell-Selective Adeno-Associated Virus-Mediated SCN1A Gene Regulation Therapy Rescues Mortality and Seizure Phenotypes in a Dravet Syndrome Mouse Model and Is Well Tolerated in Nonhuman Primates. Hum Gene Ther. June 2022; 33(11-12): 579–597. 33.

9. Laux L, Sullivan J, Perry MS, Brunklaus A, Desurkar A, Schreiber JM, et al. Zorevunersen in Children and Adolescents with Dravet Syndrome. New England Journal of Medicine. 2026 Mar 5;394(10):969–82. doi:10.1056/NEJMoa2506295