PROJECT

Supervisor:	__Gaia Colasante_____
Title:	Toward a mutation agnostic approach to treat Dravet Syndrome
Curriculum:	_____NEN_____
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 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)

Background/gap of knowledge

Dravet syndrome (DS) is a severe developmental and epileptic marked by drug-resistant seizures and a range of behavioral abnormalities, including autistic traits, intellectual disability, and cognitive impairment. It is caused by loss-of-function (LoF) mutations affecting *SCN1A* gene, leading to haploinsufficiency of the encoded alpha subunit of the voltage-gated sodium channel Nav1.1[1,2]. Current gene therapy approaches aim to compensate for *SCN1A* haploinsufficiency by enhancing expression of the functional allele at both transcriptional and post-transcriptional levels[3–10]. We recently identified a novel mechanism involving regulatory elements within the 5′ untranslated region that modulate the efficiency of *SCN1A* mRNA translation. We set an Adenine Base Editing (ABE)[11] strategy to disrupt that regulation directly in *Scn1a* gene locus that was sufficient to increaser Nav1.1 protein level and to provide therapeutic effect in pre-symptomatic DS mice.

The ABE strategy has proven highly efficient in inducing the desired edits, unintended modifications (by-stander mutations) have also been observed. In addition, there is currently no evidence that the strategy can be efficacious also in post-symptomatic animals. Furthermore, the relevance of the mechanism we are targeting remains unclear.

Rationale and hypothesis

We hypothesize that the editing strategy can be optimized using novel technologies that avoid by stander mutations and that the optimized treatment can be efficacious also in post-symptomatic DS mice.

Objectives and Specific Aims

The project will be structured around the following aims:

1) Development and optimization of the editing strategy: To improve precision of the editing tool, the PhD candidate will develop a Prime Editing (PE)[12] strategy to disrupt the identified regulatory mechanism. Both ABE and PE strategies will be directly compared for efficiency and specificity to select the best performing.

2) In vivo delivery using clinically relevant AAV vectors: the selected gene-editing strategy will be delivered by clinically relevant Adeno Associated Vectorinpost-symptomatic DS mice, and its effects on their characteristic phenotype will be assessed.

3) Investigation of physiological relevance of the regulatory mechanism: the PhD candidate will investigate the biological significance of the regulatory mechanism being targeted, with a particular focus on determining when and under which conditions this specific ion channel regulation occurs.

Expected Outcomes

The PhD candidate will evaluate the efficacy of a promising, mutation-agnostic gene-editing strategy in symptomatic Dravet mice. We anticipate achieving a dose-dependent amelioration of key disease phenotypes, including seizure frequency, survival, and behavioral deficits. In addition, we expect to gain insight into the physiological relevance of the targeted regulatory mechanism, which is also critical for assessing the safety of the gene-editing approach.

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 and prime editing strategies. He/she will learn about the most clinically relevant AAV variants and to deliver them in murine animal models. Moreover, he/she will learn to design and perform experiments related to the phenotypic assessment of Dravet mice, including surgery for implant of ECoG-transmitters, ECoG activity recording and analysis and behavioral tests.

References (max. 15)

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