The undersigned

SURNAME ____Colasante_____

FIRST NAME ____Gaia_____ born in _____*Foggia* __

Prov. *FG*_____ on _02 / _12__ / 1981

Unit: __*Stem Cell and Neurogenesis*

Residency/Postgraduate *School*[1]:

_____

Email address: _____*colasante.gaia@hsr.it*_____

Role:

☐ Vita-Salute San Raffaele University Professor/Lecturer

☐ Vita-Salute San Raffaele University Researcher/Lecturer

☐ Group Leader of the hospital site _____

☒ Project Leader of the hospital site _____

☐ Other

_____

I hereby declare that, within the framework of the PhD Course for which I wish to submit the project described below:

☒ I am already a Supervisor;

☐ I am applying for the first time as a Supervisor (CV attached);

☐ I am applying as a Supervisor as three years have elapsed since my last Application as Supervisor and the submission of a research project (CV attached).

I further declare that (select the applicable option(s)):

☐ although I am less than four years away from retirement as a university professor/researcher, I will hold a documented institutional role at the hospital _____, for at least one year beyond the official duration of the course.

☐ I serve as Supervisor for no. __ PhD candidates enrolled at other universities and I comply with the University requirement regarding the maximum number of five PhD candidates that may be supervised.

[1] To be indicated only for research projects associated with the Physician Scientist programme

I would like to present a project:

☒ **With a duration of three years**

☐ **With a duration of two years within the Physician Scientist (PhS) programme**

as part of the PhD course in:

☐ Molecular Medicine

PhD Curriculum:

☐ Basic and Applied Immunology and Oncology

☐ Cell and Molecular Biology

☐ Clinical and Experimental Medicine

☐ Neurosciences and Experimental Neurology

☒ Gene and Cell Therapy

☐ Cognitive and Behavioural Sciences

The project consists in:

1. Basic Research ☐
2. Translational Research ☐
3. Basic/ Translational research using animal models ☒
4. Clinical research ☐
5. Clinical research involving interaction with patients ☐

If items 2 and/or 3 is/are selected, I declare that

☐ I HAVE OBTAINED the approval of the responsible Institutional Animal Care and Use Committee-IACUC number _____

☒ I <u>HAVE NOT YET OBTAINED the approval</u> of the responsible Institutional Animal Care and Use Committee-IACUC (IACUC submitted, under revision)

If items 4 and/or 5 is/are selected, I declare that the project:

☐ **<u>HAS NOT YET OBTAINED</u>** <u>approval from the Ethics Committee (EC)</u>

☐ **HAS OBTAINED**, or is part of a broader study that has obtained, approval from the Ethics Committee (EC); study code and date _____

If items 4 and/or 5 is/are selected, I declare that the project:

- ☐ **HAS NOT OBTAINED** the resolution of the Institution
- ☐ **HAS OBTAINED** the resolution of the Institution on _____

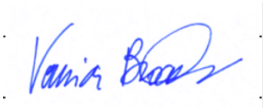
I further declare (select the applicable option(s)):

- ☐ that I have the availability of the funds necessary to finance a scholarship for the proposed project and I confirm that I have contacted the Doctoral Office regarding the management of the administrative procedures related to the funding;
- ■ that I have the availability of funds to support the research (i.e. funds for materials, reagents, and instruments required for research activities);
- ☐ that, in the case of a clinical research project, it will include a basic or translational research component to be carried out in a laboratory to be specified in the research plan, whose head will act as co-supervisor.
- ■ that I have adequate workspace and a permanent workstation available for the PhD candidate who will be selected to carry out the project;
- ■ that the proposed project can be reasonably completed within the three-year legal duration of the programme;
- ■ that the PhD student, within the activities of the relevant PhD program, will carry out only their specific doctoral project;
- ■ that the PhD student will be the first author/author of the main publication resulting from his/her project and of all publications (also after graduation) that are mainly based on his/her experimental work;
- ■ that, in the event that the PhD student is not the recipient of a UniSR grant (i.e. has won a position without a grant), I am willing to cover the cost of their scholarship with funds at my disposal. I am aware that the grant must not amount to less than the minimum required by the Ministerial Decree of 23 February 2022, amounting to € 16,243 gross per year, for three years;
- ☐ that the study is co-funded by an industrial partner or that a commercial exploitation of the findings resulting from the project's research activity is conceivable, with a potential delay in the publication of the results. I therefore commit to promptly inform potential candidates of such circumstances.

Signature of the Supervisor ____ Gaia Colasante ____ *Date* ____*28/03/2026*____

When applicable:

Group Leader Prof. /Dr. _____Vania Broccoli____

Signature ____  _____ *Date* ___*28/3/2026*__

Please note that the information provided on the following pages (unless otherwise indicated) will be made public on the University website. Therefore, it is important not to include confidential information, in compliance with any confidentiality obligations towards third parties and to protect the potential patenting of such information. For any questions, please consult the PhD Office.

PROJECT

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 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 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.

Rationale and hypothesis

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)

1. Brunklaus A, Ellis R, Reavey E, Semsarian C, Zuberi SM. Genotype phenotype associations across the voltage-gated sodium channel family. J Med Genet. 2014;51(10):650-658. doi:10.1136/jmedgenet-2014-102608

2. Catterall WA. Dravet syndrome: a sodium channel interneuronopathy. Curr Opin Physiol. 2018;2:42-50. doi:10.1016/j.cophys.2017.12.007

3. Hsiao J, Yuan TY, Tsai MS, et al. Upregulation of Haploinsufficient Gene Expression in the Brain by Targeting a Long Non-coding RNA Improves Seizure Phenotype in a Model of Dravet Syndrome. EBioMedicine. 2016;9:257-277. doi:10.1016/j.ebiom.2016.05.011

4. Colasante G, Lignani G, Brusco S, et al. dCas9-Based Scn1a Gene Activation Restores Inhibitory Interneuron Excitability and Attenuates Seizures in Dravet Syndrome Mice. Mol Ther. 2019;28(1). doi:10.1016/j.ymthe.2019.08.018

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

6. Lim KH, Han Z, Jeon HY, et al. Antisense oligonucleotide modulation of non-productive alternative splicing upregulates gene expression. Nat Commun. 2020;11(1). doi:10.1038/s41467-020-17093-9

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

The information below will not be displayed on the University website in the description of the projects offered for the academic year, and will be used for internal project assessment only.

Experimental plan (Between 2,000 and 3,000 characters including spaces):

To be completed for all types of projects; however, for CLINICAL PROJECTS, please specify:

1. *If Observational prospective, cross-sectional, or retrospective) or retro/prospective, quality of life, pharmacological, pathophysiology, genetics, epidemiological, registry/data collection, biobank, diagnostic accuracy, in vitro diagnostic device (IVD), nutraceutical/supplement, appropriateness; OR interventional (pharmacological, surgical, procedure, or medical device, and if a drug will be used, indicate the phase – I, II, III, or IV);*
2. *If a drug will be used, specify whether it has a marketing authorisation (MA), whether it will be used according to the MA or whether it does not have a MA;*
3. *If the study does not regard a drug, specify what will be studied (e.g. medical device, surgical procedure, diagnostic procedure, food supplement, etc.). If the study will use a medical device, please specify: whether it is CE marked. If CE marked, please indicate whether it will be used according to the approved use or for a new use.*
4. *Indicate the laboratory on which you intend to rely for the basic or translational part.*

The overall aim of this project is to develop a gene editing strategy to enhance the expression of alternative voltage gated sodium channels and compensate for the haploinsufficiency of *SCN1A*, which underlies Dravet syndrome. Differently from other approaches that aim to restore the proper Nav1.1 channel level by enhancing gene expression, this approach seeks to exploit and strengthen endogenous compensatory mechanisms that are naturally present during early development, such as those involving other *SCN* family members. Among them, the best candidate is *SCN3A*, thought to exert *SCN1A* function during foetal development. We have an ongoing project aiming to better elucidate the functional interaction between *Scn1a* and *Scn3a.* We have identified conserved regulatory motifs with repressive functions on the translation, specifically of upstream open reading frames (uORFs) and validated that disruption of those uORFs can enhance translation of the main ORF of different *SCN* genes.

1) The first goal of the PhD student will be optimizing the editing strategies already available in the lab disrupting uATGs of uORFs in other *SCN* genes. The candidate will compare available Adenine Base Editing (ABE) strategy efficiency with Prime editing (PE) by testing them in wild type primary neurons derived from mice. Both efficiency and specificity will be evaluated together with their ability to enhance the corresponding Nav channel expression at protein level. This will be achieved by NGS analysis at target sequence and WB/Automatic WB (Jess) quantification.

2) The second objective aims to determine which alternative Nav can most effectively compensate for Nav1.1 deficiency. To address this, the selected ABE/PE strategies will be applied to primary neurons derived from a DS mouse model ($Scn1a^{+/-}$) to assess functional outcomes. Electrophysiological recordings will be used to examine the firing properties of inhibitory interneurons, which are particularly affected in the disease, while network-level activity will be

evaluated using multi-electrode array recordings. By systematically comparing different the effect of the enhancement of different *SCN* paralogs, such as *SCN2A, SCN3A, and SCN8A*, it will be possible to identify the channel that most effectively restores neuronal excitability and network balance.

3) The final objective will focus on validating the selected strategy in disease models. In vivo delivery of the optimized editing system will be performed in Dravet syndrome mouse models, ideally during an early postnatal window, to assess its impact on survival, seizure frequency, and behavioral phenotypes. In parallel, the approach will be tested in human induced pluripotent stem cell (iPSC)-derived neurons from patients, to confirm its relevance in a human context and specifically on hiPSCs derived from patients carrying DN mutations.

Available methods and experimental models (max. 600 characters including spaces):

To be completed for all types of projects; however, for CLINICAL PROJECTS, please specify:

1. *whether participants (patients and/or healthy volunteers) will be recruited;*
2. *whether biological samples will be taken from participants (patients and/or healthy volunteers);*
3. *whether the biological samples will be stored in a Biobank (specify which Biobank);*
4. *whether biological samples are already stored and available in a Biobank (specify which Biobank);*
5. *whether biological samples or data will be collected in addition to those already included in the routine standard of care from routine practice (specify type of samples/data, quantity and timing);*
6. *whether procedures will be required in addition to those already included in the routine standard of care from routine practice (e.g. Consultations, laboratory tests, clinical/instrumental examinations). Specify the additional procedures, quantity and timing).*

The lab has already established experience in gene editing technology, including BE and PE. In addition, we have ongoing collaborations (Dr. Gerald Schwank, University of Zurich and Dr Julian Grunewald, University of Munich) for their optimization. Relevant disease models of DS, including hiPSC cell lines from DS patients and mouse models are available in the lab. We have expertise in the phenotypic characterization of DS models using different readouts, including neuronal activity, seizures and behavioral alterations.

Role of the PhD student (max. 600 characters including spaces):

The PhD candidate will share responsibility for the project with the Supervisor (DOS) and is expected to dedicate the majority of his/her time and effort to it. In addition to learning the main techniques employed and necessary to carry out the project, he/she will progressively be expected to identify problems and propose potential solutions for discussion with the Supervisor.

In other words, he/she is the primary contributor to the project. He/she will also be asked to contribute to manuscript preparation at the project's conclusion.

Impact of the expected results in the field of research (max. 600 characters including spaces):

DS is a devastating genetic disorder with an estimated incidence between 1 in 20,000 and 1 in 40,000 live births characterized by severe seizures and cognitive impairment. While two gene therapies are currently in clinical trial, patients carrying DN mutations are excluded from that, as the therapy upregulate both healthy and wt allele. If successful, our project will lead to the development of the first gene therapy strategy suitable also for patients affected by DN mutations.

In the case of clinical research, include the timeline for the project approval process up to the authorizing resolution of the Institution.

Period of attendance at a foreign institution

Mandatory for the PhD course in Cognitive and Behavioral Sciences

The PhD course in Cognitive and Behavioral Sciences encourages attendance at foreign universities and research institutes, promoting the acquisition of advanced skills and methodologies in international contexts.

Please indicate whether a period of activity at a foreign institution is planned. If so, specify:

- *Host institution (name of the University/Institute and country)*
- *Duration of stay (not less than 3 months)*
- *Integration with the research project (describe how this experience will contribute to the objectives of the proposed project)*

The information provided is not binding and may be subject to modifications based on the project's development and available opportunities.

For the use by the PhD Office

FOR OPINION – *(ONLY for Programs divided into Curricula)*

Signature of the Curriculum Supervisor _____ *Date* _____

FOR APPROVAL

Signature of the PhD Course Coordinator _____