PROJECT

Supervisor:	Alessandro Sessa
Title:	**Gene Therapy approaches for *SETBP1* Haploinsufficiency Disorder**
Curriculum:	NEN
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/neuroscience/neuroepigenetics.html

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

Background/gap of knowledge

SETBP1 haploinsufficiency disorder (SHD) is a rare neurodevelopmental disorder characterized by symptoms such as developmental delay, intellectual disability, language disorder, and behavioral impairments (1). Mechanistic explanations and effective treatments are missing.

The disease arises from mutations in one SETBP1 allele leading to reduced expression levels (2). Thus, gene therapy (GT) holds promise as a potential treatment. However, our knowledge of SETBP1 is far from comprehensive, especially on when and how it plays a pivotal role during brain development and function (3). Similarly, the feasibility and extent of symptom reversal in SHD remain unknown. Furthermore, potential side effects due to elevated levels of the "therapeutic" SETBP1 protein exist. Indeed, the accumulation of SETBP1 is the cause of the Schinzel-Giedion syndrome (SGS), a devastating disease which symptoms include severe developmental delay, progressive brain atrophy, and frequent drug-resistant seizures (4,5). As for SHD, in SGS the extent of the postnatal impact of the pathogenic insult is completely unestimated, therefore the accumulation of SETBP1 in mature brain cells, as might happen with excessive GT in SHD, may be either inherently dangerous or not.

Rationale and hypothesis

Here, we propose experimental lines aiming to gain fundamental knowledge on pathophysiological mechanisms involving SETBP1. In our vision, this prepares the ground on which future GT attempts for SHD.

Specifically, we hypothesize that a balanced SETBP1 expression in mature neuronal cells is critical for their correct functionality, while the accumulation needs to happen during neural

development to be damaging. This would represent an important "go" signal for GT attempt for SHD.

Objectives and specific aims

We address this hypothesis by leveraging 2 unique in house-generated murine models with the ability to manipulate SETBP1 levels. One model permits to normalize the level of *Setbp1* in SHD on-demand, while in the other SETBP1 can be spatially and temporally overexpressed through Cre-recombination (6).

The following two aims are propaedeutic for future GT approaches for SHD.

1: Evaluation of phenotypic recovery upon Setbp1 reactivation in SHD model in vivo.

2: Risk-assessment of SGS-like SETBP1 accumulation in postnatal brain.

Expected outcomes

We expect to achieve either SETBP1 reactivation or pathological overexpression, starting from either perinatal or juvenile stages. Given the phenotypes of the two opposite disorders and our preliminary data, we envisage molecular, morphological, behavioral and electrophysiological characterization of the "treated" models. We will gain information on the rescue of murine pathology which occurs after Setbp1 haploinsufficiency and eventual detrimental effects.

The proposed assessment will provide an exhaustive snapshot of GT possibility to achieve the reversion of the SHD symptoms and the evasion of SGS-like risks.

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

The project aims to gain functional characterization of SETBP1 manipulation both at the level of cell-autonomous behaviour up to the organization of neuronal circuitries. The student will perform, molecular investigation of SETBP1 activity (transcriptomics, epigenetic rewiring)(6), morphological inspection of the brain as well as electrophysiological recording (in collaboration with Dr. S. Taverna, OSR - Division of Neuroscience).

The student will also learn how to manage colonies of GM animals and prepare them for behavioural studies that will be performed within the OSR facility.

References (max. 15)

1. Jansen NA et al. Clinical delineation of SETBP1 haploinsufficiency disorder. Eur J Hum Genet. 2021 Aug;29(8):1198-1205. doi: 10.1038/s41431-021-00888-9.

2. Filges I et al. Reduced expression by SETBP1 haploinsufficiency causes developmental and expressive language delay indicating a phenotype distinct from Schinzel-Giedion syndrome. J Med Genet. 2011 Feb;48(2):117-22. doi: 10.1136/jmg.2010.084582.

3. Antonyan L, Ernst C. Putative Roles of SETBP1 Dosage on the SET Oncogene to Affect Brain Development. Front Neurosci. 2022 May 24;16:813430. doi: 10.3389/fnins.2022.813430

4. Piazza R et al. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet. 2013 Jan;45(1):18-24. doi: 10.1038/ng.2495.

5. Piazza R et al. SETBP1 induces transcription of a network of development genes by acting as an epigenetic hub. Nat Commun. 2018 Jun 6;9(1):2192. doi: 10.1038/s41467-018-04462-8.

6. Zaghi M et al. Balanced SET levels favor the correct enhancer repertoire during cell fate acquisition. Nat Commun. 2023 Jun 3;14(1):3212. doi: 10.1038/s41467-023-39043-x.