

 <p>UniSR Università Vita-Salute San Raffaele</p>	<p>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</p>	<p>MO 20-5 ed. 01 del 21/02/2025 PO 20 Page 4 of 10</p>
---	--	--

PROJECT

Supervisor: Laura SILVESTRI

Title: Targeting the Hepatic BMP-SMAD Pathway in Leptin Receptor Deficiency (LEPRD): A New Strategy for Treating Severe Pediatric Obesity

Curriculum: Cellular and Molecular Biology

Link to the personal page of the University or relevant hospital site website: <https://research.hsr.it/en/divisions/genetics-and-cell-biology/regulation-of-iron-metabolism/laura-silvestri.html>

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

Background/gap of knowledge

Leptin receptor deficiency (LEPRD) is a rare genetic disorder causing early-onset obesity, hyperglycemia, and insulin resistance due to impaired leptin-melanocortin signaling, leading to abnormal feeding behavior. Standard obesity treatments are often ineffective or contraindicated due to persistent hyperphagia¹.

Setmelanotide (SET), an MC4R agonist, is approved for rare monogenic obesity, including LEPRD, as it bypasses the defective leptin receptor. While it reduces hunger, weight loss in LEPRD patients is less marked than in other forms of monogenic obesity^{2,3}. Additionally, some patients had to pause or lower the dose due to side effects^{4,5}. Thus, further therapeutic strategies are needed to improve its efficacy.

Rationale and hypothesis

TMPRSS6, a protease in hepatocytes, inhibits the BMP-SMAD pathway. Tmprss6-KO mice on a high-fat diet (HFD) showed reduced adipose tissue and improved insulin resistance⁶, indicating hepatic Tmprss6 deletion alters metabolism. Preliminary data suggest that reducing Tmprss6 in hepatocytes lowers weight gain, liver steatosis, and adipocyte hypertrophy in HFD mice. In the LEPRD mouse model, Tmprss6 downregulation also reduces hepatosteatosis, adipocyte hypertrophy, and inflammation, positioning TMPRSS6 as a key factor in this genetic obesity.

The project's central hypothesis is that TMPRSS6 is a novel regulator of lipid and glucose metabolism and a promising pharmacological target for LEPRD, either alone or combined with



UniSR

Università Vita-Salute
San Raffaele

**APPLICATION TO ACT AS SUPERVISOR AND
RESEARCH PROJECT PROPOSAL**

MO 20-5
ed. 01 del 21/02/2025
PO 20
Page 5 of 10

SET to enhance treatment efficacy. The availability of a well-established siRNA-based method for *TMPRSS6* downregulation in hepatocytes offers a potential new therapeutic approach for this severe genetic obesity.

Objectives and specific aims

The objectives of the project are:

1. To investigate whether (AIM 1) and how (AIM 3) the pharmacological upregulation of *Tmprss6*, by enhancing the hepatic BMP-SMAD pathway, improves LEPRD. To achieve this, we will utilize db/db mice, which develop LEPRD, and treat them with GalNAC-modified *Tmprss6* siRNA for selective hepatocyte targeting.
2. To demonstrate that the combined targeting of MC4R by SET and *Tmprss6* by siRNA is more effective than SET alone in improving the LEPRD phenotype and enhancing the efficacy of low-dose SET, thereby reducing the risk of adverse events (AIM 2).

Expected outcomes

1. The hepatocytic BMP-SMAD pathway, which is diminished in LEPRD, plays a crucial role in regulating lipid and glucose metabolism. Its selective upregulation in hepatocytes through *TMPRSS6*-siRNA treatment counteracts weight gain, liver steatosis, adipocyte hypertrophy, inflammation, and lowers glycemia.
2. The efficacy of Setmelanotide can be enhanced by combining it with *TMPRSS6*-siRNA treatment.
3. Hepatokines, whose expression is modulated by the improvement of the LEPRD phenotype following *TMPRSS6* downregulation and BMP-SMAD pathway activation, may serve as potential autocrine and endocrine regulators of lipid and glucose metabolism. These hepatokines could exert autocrine or paracrine effects on metabolically active tissues, such as adipose tissue and skeletal muscle, both of which are compromised in LEPRD.

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

- Management of the db/db mouse colony
- Evaluation of the LEPRD phenotype: GTT/ITT, organ weights, and full phenotypic characterization
- Design of an experimental approach to reduce *Tmprss6* expression using siRNA (and treatment with SET in combination)



- Establishment and treatment of primary cell cultures with selected hepatokines (hepatocytes, adipocytes, myoblasts)
- Critical evaluation of the obtained results
- Presentation of data at a conference
- Writing a research paper

References (max. 15)

1. Fitch, A. K., et al. (2024). Differentiating monogenic and syndromic obesities from polygenic obesity: Assessment, diagnosis, and management. *Obesity Pillars*, 11, 100-110.
2. Clément, K., et al. (2020). Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials. *The Lancet Diabetes & Endocrinology*, 8(12), 960-970.
3. Loos, R. J. F., & Yeo, G. S. H. (2022). The genetics of obesity: from discovery to biology. *Nature Reviews Genetics*, 23(2), 120-133.
4. Argente J, Verge CF, Okorie U, Fennoy I, Kelsey MM, Cokkinias C, Scimia C, Lee HM, Farooqi IS. Setmelanotide in patients aged 2-5 years with rare MC4R pathway-associated obesity (VENTURE): a 1 year, open-label, multicenter, phase 3 trial. *Lancet Diabetes Endocrinol*. 2025 Jan;13(1):29-37.
5. Clément K, van den Akker E, Argente J, Bahm A, Chung WK, Connors H, De Waele K, Farooqi IS, Gonneau-Lejeune J, Gordon G, Kohlsdorf K, Poitou C, Puder L, Swain J, Stewart M, Yuan G, Wabitsch M, Kühnen P; Setmelanotide POMC and LEPR Phase 3 Trial Investigators. Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials. *Lancet Diabetes Endocrinol*. 2020 Dec;8(12):960-970.
6. Folgueras, A. R., et al. (2018). Matriptase-2 deficiency protects from obesity by modulating iron homeostasis. *Nature Communications*, 9(1), 1350.