AZIENDA OSPEDALIERO---UNIVERSITARIA
"MAGGIORE DELLA CARITÀ" UNIVERSITÀ DEL
PIEMONTE ORIENTALE
SCDU di Endocrinologia

Direttore: Prof. Paolo Marzullo

PROTOCOL

TITLE

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

Version 2 (20/03/2026)

SCDU di Endocrinologia
AOU Maggiore della Carità, Via Mazzini 18,
Padiglione G, 28100 Novara Tel. 03213733209
(Reparto);; Fax. 03213733905
endocrinologia@maggioreosp.novara.it

STUDY BACKGROUND

Leptin receptor deficiency (LEPRD) is an extremely rare autosomal recessive endocrine disorder (estimated prevalence between approximately 1 in 1,000,000 and 1 in 100,000). The condition is characterized by severe early-onset obesity associated with hyperphagia, hyperglycemia, hyperinsulinemia, insulin resistance, and pituitary hormone deficiencies (1). The primary pathophysiological defect lies in the impairment of the leptin–melanocortin signaling pathway, which leads to a severe dysregulation of eating behavior.
Alterations in appetite and metabolism are not unique to leptin receptor deficiency (LEPRD), but are also observed in genetic lipodystrophy syndromes associated with leptin deficiency (generalized or partial forms) (2). In these conditions, there is a significant loss of adipose tissue along with impaired expression and function of leptin and its receptor in peripheral tissues, such as skeletal muscle and residual adipose tissue. This defect interferes with the regulation of energy homeostasis and contributes to metabolic dysfunction, including alterations in glucose and lipid levels, as well as ectopic hepatic fat accumulation.
Conventional treatments for obesity—including caloric restriction, pharmacotherapy, and bariatric surgery—do not yield durable benefits, as they fail to correct the severe underlying hyperphagia driving the disease.

Setmelanotide (SET) is a selective melanocortin-4 receptor (MC4R) agonist recently approved by the FDA and AIFA for specific forms of monogenic obesity, including leptin receptor deficiency (LEPRD) (3). The drug acts downstream of the leptin–melanocortin pathway, bypassing the receptor defect and partially restoring signaling, which leads to a significant reduction in hunger. However, in patients with LEPRD, the weight loss achieved is generally lower than that reported in other monogenic obesity syndromes.
Similarly, metreleptin is approved for certain forms of genetic lipodystrophy, but its effect on metabolic and hepatic parameters remains incomplete (4). Therefore, an unmet clinical need persists for therapeutic strategies capable of both counteracting disease progression and its metabolic complications, and enhancing the efficacy of SET and metreleptin.
TMPRSS6 is a type II transmembrane serine protease selectively expressed by hepatocytes. It inhibits hepatic BMP–SMAD signaling by cleaving the coreceptor hemojuvelin (HJV) and other components of the cascade, thereby reducing hepcidin synthesis. In murine models, genetic inactivation of *Tmprss6* or its silencing via antisense oligonucleotides leads to constitutive activation of the BMP–SMAD pathway in hepatocytes, accompanied by significant metabolic effects (improvement in glucose metabolism, reduction in adipose tissue accumulation, and attenuation of inflammation) (5,6).

Taken together, these findings suggest that modulating TMPRSS6 may represent an innovative therapeutic strategy for patients with LEPRD or leptin deficiency (partial or generalized forms).

AIMS OF THE STUDY

Preliminary studies in murine models have identified specific hepatokines—molecules produced by the liver that regulate systemic metabolism—with particular reference to the TMPRSS6 enzyme, which is involved in alterations of the leptin-receptor axis. TMPRSS6 exerts both hepatic effects, by improving lipid metabolism, and systemic effects, acting on adipose tissue and skeletal muscle. In humans, rare diseases such as leptin

receptor deficiency (LEPRD) and genetic forms of lipodystrophy with leptin deficiency display alterations in glucose and lipid metabolism, often associated with hepatic steatosis.

Based on this preclinical evidence, the primary objective of this human study is to validate the findings obtained in mouse models. To this end, serum and plasma samples will be collected from patients with LEPRD or genetic lipodystrophy to quantify specific circulating markers previously identified in preclinical studies as being regulated by TMPRSS6. This will allow us to determine whether the metabolic alterations observed in animal models are also present in humans.

This integrated approach will enable the identification of a specific hepatic metabolic signature associated with TMPRSS6 and assess whether this molecular mechanism could represent a novel therapeutic target for managing metabolic (glycemic and lipidic) and hepatic alterations in patients affected by these rare conditions.

STUDY DESIGN

This is a pilot, observational, cross-sectional study. The target population consists of pediatric or adult patients diagnosed with LEPRD or genetically-based leptin deficiency (generalized or partial lipodystrophy). The study is conducted within the framework of the Fondazione Telethon grant, in collaboration with the Università Vita-Salute San Raffaele (Multi-Round Call 2025).

SUBJECTS AND METHODS

We plan to recruit approximately 35 subjects with generalized or partial lipodystrophy currently under follow-up at the Complex University Structure (SCDU) of Endocrinology, AOU "Maggiore della Carità" in Novara. Regarding patients with LEPRD, given the extreme rarity of the condition, it is not possible to estimate the number of enrollable subjects *a priori*; therefore, consecutive enrollment of eligible patients presenting to our center will be adopted, targeting a maximum of 10 subjects.

The study will have an overall duration of 36 months, aligning with the timelines specified in the Fondazione Telethon call, and will commence in September 2026.

Inclusion Criteria

- Male and female patients, aged 0 to 99 years
- Patients diagnosed with leptin receptor deficiency (LEPRD) or genetic lipodystrophy with leptin deficiency (partial or generalized forms)
- Untreated or treated with setmelanotide or metreleptin

Exclusion Criteria

- Other types of monogenic or genetic obesity

- Confirmed or planned pregnancy during the months of study participation
- Hepatic insufficiency due to other causes (e.g., alcohol consumption)
- Genetic dyslipidemia due to other causes

Following review of the Study Information Sheet (see Informed Consent Management section) and an adequate informative interview with the research staff, participants will be enrolled only after providing free and voluntary consent, formalized by signing the Informed Consent form.

Study Duration

The study will last 36 months, corresponding to the timeline of the Fondazione Telethon grant (Multi-Round Call 2025). During this period, eligible subjects will be recruited according to the inclusion criteria, biological samples and clinical data will be collected, and laboratory analyses and data processing will be conducted.

Recruitment Strategy

During routine clinical visits, potentially eligible subjects meeting the inclusion criteria will be informed about the opportunity to participate in this study. The research staff will explain the aims, methods, and implications of participation, review the Information Sheet, and answer any questions. Participants (and, in the case of minors, their parents/legal guardians along with the minor according to their age and level of understanding) will be given adequate time to evaluate the proposal, consult with family members or healthcare providers, and decide freely without any pressure. Enrollment will take place exclusively after obtaining written informed consent and, where applicable, minor assent.

Blood Sampling Procedures and Data Collection

Following the acquisition of informed consent, operational details for blood sampling will be arranged with the participant. Blood collection will take place at our center concurrently with routinely scheduled clinical blood draws. On that occasion, the participant must present in a fasted state according to clinical instructions, and two additional blood tubes (total volume 6 mL) will be collected for serum and plasma extraction.

Samples will be coded with a unique identifier and transferred to the UPOBiobank for processing and storage according to standard operating procedures. Strictly necessary clinical data (e.g., anthropometric parameters, diagnosis, and routine blood test results) will, upon consent, be recorded on the REDCap platform with controlled access. These data will subsequently be integrated with the results of the biological sample analyses for research purposes.

Analysis of Biological Samples

Biological samples stored at the UPOBiobank will be requested at the end of the recruitment phase, or upon reaching the target sample size, for the quantification of hepatokines in serum and plasma. Analyses will be performed on coded and anonymized samples. Quantification may be conducted using enzyme-linked

immunosorbent assays (ELISA) or multiplex assays. Dedicated locations for laboratory assays will be the Laboratory of Endocrinology and Nutrition (located at Palazzo Bellini, Via Solaroli 17, Novara) or the UPO-CAAD (Via Giovanni Bovio 17, Novara).

Statistical Considerations

Given the pilot, observational nature of the study and the extreme rarity of the condition under investigation, the sample size will be limited. Therefore, analyses will be primarily descriptive, aimed at characterizing the distribution of the parameters of interest. Summary statistics such as mean and standard deviation, or median and interquartile range (depending on data distribution), will be reported alongside proportions and corresponding confidence intervals where appropriate.

Exploratory analyses comparing patient groups or subgroups regarding serum hepatokine levels and clinical variables may be conducted. Qualitative evaluations and assessments of biological plausibility with respect to results obtained in mouse models and primary human cells (analyzed at the collaborating center responsible for the preclinical phase) will also be performed. Data will be managed and analyzed using appropriate statistical software (Stata; R) with a focus on data consistency and quality. Formal confirmatory inferential hypothesis testing will not be conducted, given the limited sample size.

Expected Results

The study aims to validate in patients with LEPRD or genetic lipodystrophy with leptin deficiency the findings observed in animal models before and after TMPRSS6 modulation. If human data prove consistent with preclinical results, TMPRSS6 inhibitors could be evaluated in subsequent clinical trial phases for these target diseases.

ETHICAL ASPECTS

The clinical study protocol will be submitted for review and approval to the competent Inter-hospital Ethics Committee of Novara prior to the initiation of any research activity. The study will also be registered on ClinicalTrials.gov before the start of enrollment. The research will be conducted in compliance with the principles of human subject protection, risk minimization, and proportionality to expected benefits.

All potential participants will receive adequate, comprehensive, and understandable information; participation will be free and voluntary, occurring only after comprehension and signing of the informed consent form. Participants may withdraw consent at any time without providing a reason and without affecting their routine clinical care.

The study will be conducted in accordance with:

- The Ethical Principles for Medical Research Involving Human Subjects (Declaration of Helsinki – World Medical Association, current version);
- Good Clinical Practice guidelines (ICH/GCP) of the European Union;

- The Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (Oviedo Convention of 04/04/1997); Italian Codes of Ethics for Medical Professions; and applicable national legislation regarding clinical studies;
- Applicable regulations on personal data protection (EU Regulation 2016/679 – GDPR and national legislation).

Management of Informed Consent

An Information Sheet is available detailing the study design, objectives, procedures, sample and data collection, potential benefits, and risks. This information will be presented to potential participants, who will have full freedom to give consent or withdraw at any point during the study.

Management of Sensitive Data

The following European and national regulations are applied:

- Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 (GDPR);
- Italian Legislative Decree 30 June 2003, n. 196 (Personal Data Protection Code), as amended by Legislative Decree 10 August 2018, n. 101;
- Italian Data Protection Authority (Garante Privacy) measures regarding the processing of special categories of data pursuant to art. 21, paragraph 1, of Leg. Dec. n. 101/2018.

DATA AND BIOLOGICAL SAMPLE HANDLING

The study involves collecting biological samples and clinically relevant data (anthropometric parameters, diagnosis, and relevant routine blood tests) for research purposes. Biological samples and associated personal/sensitive data will be immediately pseudonymized through the random assignment of unique alphanumeric study codes.

Clinically relevant data will be entered into the REDCap electronic data capture system using the same unique code. The key linking codes to subject identities will be stored on password-encrypted personal devices accessible only to the Principal Investigator and authorized co-investigators. Therefore, connecting analytical data back to a specific individual will only be possible for authorized research staff when strictly necessary (e.g., for participant health reasons).

Personal data will be processed according to the principles of lawfulness, fairness, transparency, purpose limitation, data minimization, and accuracy (Art. 5 GDPR) in electronic and paper format by authorized personnel. Availability, management, access, storage, and usability of data are secured through technical and organizational measures ensuring appropriate security levels (Arts. 25 and 32 GDPR). Access is limited to researchers involved in the study and authorized by the Principal Investigator.

Biological samples will be stored at the UPO Biobank under certified quality and safety standards. Samples will be pseudonymized, and only authorized UPO Biobank staff may link the pseudonymization code to sensitive data under strict necessity. Researchers performing assays will have no means to connect sample codes to participant identities. Pseudonymized sample aliquots may be provided to facilities performing laboratory analyses; any remaining sample will be returned to UPO Biobank.

Participants will also be offered an optional, voluntary opportunity (via the "UPO Biobank Participation Agreement") to consent to the use of their biological samples for broader population studies at the UPO Biobank.

Personal data collected will be stored at the study site for 15 years following study completion, or longer if required by applicable legal obligations of the Controller. Study findings will be published or presented in scientific communications solely in an anonymous, aggregated format.

Data Controllers, within their respective responsibilities, are:

- **AOU "Maggiore della Carità" di Novara**
- **Data Protection Officer (DPO):** Dr. Alessandra Gaetano (dpo@slalomsrl.it)

COSTS / FUNDING

Study funds are provided to the Investigator through the Fondazione Telethon grant, in collaboration with Università Vita-Salute San Raffaele, Milan (Multi-Round Call 2025).

REFERENCES

1. Kleinendorst L, Abawi O, van der Kamp HJ, et al. Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics. *Eur J Endocrinol*. 2020;182(1):47-56. doi:10.1530/EJE-19-0678
2. Akinci B, Gular MC, Oral EA. Lipodystrophy Syndromes: Presentation and Treatment. In: Feingold KR, Adler RA, Ahmed SF, et al., eds. *Endotext*. South Dartmouth (MA): MDText.com, Inc.; August 21, 2024.
3. Clément K, et al. 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*. 2020;8(12):960–970. doi:10.1016/S2213-8587(20)30364-8
4. Sekizkardes H, Cochran E, Malandrino N, Garg A, Brown RJ. Efficacy of Metreleptin Treatment in Familial Partial Lipodystrophy Due to PPARG vs LMNA Pathogenic Variants. *J Clin Endocrinol Metab*. 2019;104(8):3068-3076. doi:10.1210/jc.2018-02787
5. Pettinato M, et al. Targeting the Liver Serine Protease TMPRSS6 Ameliorates Steatosis and Attenuates Fibrosis in Experimental MASLD. *Liver Int*. 2025 Jul;45(7):e70163. doi:10.1111/liv.70163
6. Folgueras AR, et al. Matriptase-2 deficiency protects from obesity by modulating iron homeostasis. *Nature Communications*. 2018;9(1):1350. doi:10.1038/s41467-018-03853-1