PROJECT

Supervisor:	SIMONINI MARCO
Title:	**Mechanistic Dissection of Biological Pathways in Salt-Sensitive Hypertension.**
Curriculum:	Cell and Molecular Biology

Link to the personal page of the University or relevant hospital site website:	https://www.unisr.it/docenti/s/simonini-marco https://www.hsr.it/dottori/marco-simonini

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

Background/gap of knowledge Hypertension (HT), a multifactorial condition influenced by genetic and environmental interplay, leads to significant morbidity and mortality. Our research focuses on mechanisms underlying salt-sensitive (SS) blood pressure (BP) regulation, with attention to the lanosterol synthase (LSS) variant rs2254524, a key player in steroid/cholesterol biosynthesis associated with SS-HT and acute kidney injury (AKI). Our knock-in (KI) mouse model $Lss^{V643L/V643L}$, harboring the corresponding substitution, develops HT accompanied by cardiac injury when exposed to a high-salt diet (HSD) for one month. Preliminary proteomic profiling after HSD indicates significant dysregulation of cholesterol metabolism and activation of inflammatory processes, driving adverse progressive organ damage. Deeper interrogation of genome-wide association (GWA) datasets across multiple hypertensive cohorts may identify genetic variants associated with poor prognosis and help stratify risk within the broader population.

Rationale and hypothesis The convergence of altered cholesterol metabolism, inflammation, and hypertensive injury observed in the Lss KI model suggests a mechanistic axis linking metabolic perturbation to immune-mediated organ damage. Defining the sequence of these events is essential for understanding how the Lss variant confers susceptibility to SS-HT and for identifying therapeutic targets. Rostafuroxin, a targeted Na^+/K^+-ATPase antagonist, will be used to test whether pharmacological intervention can rescue the altered phenotypes.

Objectives and specific aims *Aim 1*: To extend our proteomics findings, we will perform multiomic profiling in KI and wild-type mice exposed to HSD or normal salt at multiple time points. Inflammation and lipid profiles will be examined to map systemic alterations. *Aim 2*: To assess the efficacy of Rostafuroxin in counteracting HT and organ injury in HSD-exposed Lss KI mice,

and to determine whether pharmacological intervention can restore BP and halt tissue damage progression. *Aim 3*: To evaluate candidate biomarkers in human cohorts including SS individuals and hypertensive patients, assessing their ability to reflect early or advanced organ injury. GWA datasets will be analyzed to identify variants within selected pathways.

Expected outcomes

This study will define the temporal and mechanistic link between HSD, cholesterol dysregulation, and inflammation-driven organ injury in the Lss^V643L/V643L^ model. Rostafuroxin treatment will clarify whether the altered phenotype is reversible. The study will yield candidate biomarkers of salt-induced organ injury for validation in human cohorts, contributing to targeted preventive and therapeutic strategies.

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

- Molecular biology techniques (DNA, RNA extraction, qPCR, Western blotting, ELISA and histology)
- Mouse lines management (maintenance, BP measurement, blood collection, sacrifice).
- Statistical data analyses
- Genetic analyses on various human cohorts
- Independent planning and management of long-term research projects
- Lab work organization and tasks prioritizing
- Collaborative work within research teams
- Adherence to good laboratory and safety practices
- Acquisition of advanced scientific writing and communication skills
- Production of clear manuscripts, reports, and presentations

References (max. 15)

1. Lanzani C, Citterio L, Glorioso N, Manunta P, Tripodi G, Salvi E, Delli Carpini S, Ferrandi M, Messaggio E, Staessen JA, Cusi D, Macciardi F, Argiolas G, Valentini G, Ferrari P, Bianchi G. Adducin- and ouabain-related gene variants predict the antihypertensive activity of rostafuroxin, part 2: clinical studies. *Sci Transl Med.* 2010;2(59):59ra87.

2. Lanzani C, Gatti G, Citterio L, Messaggio E, Delli Carpini S, Simonini M, Casamassima N, Zagato L, Brioni E, Hamlyn JM, Manunta P. Lanosterol synthase gene polymorphisms and changes in

endogenous ouabain in the response to low sodium intake. *Hypertension.* 2016;67(2):342–348. DOI: 10.1161/HYPERTENSIONAHA.115.06415.

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4. Faienza S, Citterio L, Zagato L, Messaggio E, Lanzani CL, Simonini M, Canciani B, Sanvito F, Rampoldi L, Pavlovic D, Manunta P. A novel mouse model recapitulates the effects of rs2254524 variant in the lanosterol synthase gene on salt sensitivity and organ damage. *J Hypertens.* 2025;43(1):80–89. DOI: 10.1097/HJH.0000000000003843.

5. Simonini M, Lanzani C, Bignami E, Casamassima N, Frati E, Meroni R, Messaggio E, Alfieri O, Hamlyn J, Body SC, Collard CD, Zangrillo A, Manunta P. A new clinical multivariable model that predicts postoperative acute kidney injury: impact of endogenous ouabain. *Nephrol Dial Transplant.* 2014;29(9):1696–1701. DOI: 10.1093/ndt/gfu200.

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7. Simonini M, Casanova P, Citterio L, Messaggio E, Lanzani C, Manunta P. Endogenous ouabain and related genes in the translation from hypertension to renal diseases. *Int J Mol Sci.* 2018;19(7):1948. DOI: 10.3390/ijms19071948.

8. Bignami E, Casamassima N, Frati E, Lanzani C, Corno L, Alfieri O, Gottlieb S, Simonini M, Shah KB, Mizzi A, Messaggio E, Zangrillo A, Ferrandi M, Ferrari P, Bianchi G, Hamlyn JM, Manunta P. Preoperative endogenous ouabain predicts acute kidney injury in cardiac surgery patients. *Crit Care Med.* 2013;41(3):744–755. DOI: 10.1097/CCM.0b013e3182741599.

9. Manunta P, Hamlyn JM, Simonini M, Messaggio E, Lanzani C, Bracale M, Argiolas G, Casamassima N, Brioni E, Glorioso N, Bianchi G. Endogenous ouabain and the renin-angiotensin-aldosterone system: distinct effects on Na handling and blood pressure in human hypertension. *J Hypertens.* 2011;29(2):349–356. DOI: 10.1097/HJH.0b013e32833ea821.

10. Maj C, Salvi E, Citterio L, Borisov O, Simonini M, Glorioso V, Barlassina C, Glorioso N, Thijs L, Kuznetsova T, Cappuccio FP, Zhang ZY, Staessen JA, Cusi D, Lanzani C, Manunta P. Dissecting the polygenic basis of primary hypertension: identification of key pathway-specific components. *Front Cardiovasc Med.* 2022;9:814502. DOI: 10.3389/fcvm.2022.814502.