UNIVERSITÀ DI SIENA
1240

DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO

# Università degli Studi di Siena
# Dottorato di Ricerca in Medicina Molecolare

## Scheda Relazione Annuale Dottorando
(da redigere in lingua inglese)

*Pisa, 27/09/24*

Al Collegio Docenti del Dottorato in Medicina Molecolare

dr.ssa **Sara Bernardi**

ciclo XXXVIII tutor Dott.ssa Maria Marchese/Prof.ssa Federica Gemignani

Attività scientifica svolta nel 1°/2° anno di Dottorato, Anno Accademico **2°**

*(massimo 2000 caratteri totali, spazi inclusi)*
*descrivere brevemente la propria ricerca, suddividendola in*:

**- Introduzione**
The neuronal ceroid lipofuscinoses (NCL) are a group of rare genetic neurodegenerative disorders, predominantly occurring during childhood and early adult life, multiple studies identified different forms of NCL associated with mutations in 13 genes (*cln1-cln8*, *cln10-cln14*), characterized by accumulation of autofluorescent storage material in lysosomes. The aim of my PhD project is to develop and characterized *cln1/3/5/8*-deficient knock-out zebrafish lines to unravel new pathomechanisms behind this disease and to test molecules through high-throughput drug screening.
**- Metodiche utilizzate**
*cln1*$^{-/-}$ and *cln3*$^{-/-}$ F2 fish were screened by PCR to identify homozygous mutations obtained using CRISPR-Cas9 technology. In *cln5-/-* mutants western blot method was used to validate potential new biomarkers identified through transcriptomic and proteomic analyses. Locomotor behavioural defects were analyzed using video-tracking software (DanioScope and DanioVision). Brains from adult and juvenile zebrafish were used to extract lipids for LC/MS analysis to obtain lipidomic profiles of wild-type and mutant zebrafish.
**- Risultati ottenuti**
Screening of homozygous fish for *cln1* and *cln3* mutations is ongoing due to the low mutation rate. *cln5*$^{-/-}$ fish were deeply characterized, demonstrating that they recapitulate many disease phenotypes, such as impairment of locomotor behaviour in *cln5*$^{-/-}$ larvae compared to wild-type siblings. RNA-seq and proteomic analyses revealed many dysregulated pathways in *cln5*$^{-/-}$ fish, showing novel impaired molecular pathways such as impaired glucose metabolism and calcium signalling. Additionally, I analysed also the *cln8*$^{-/-}$ model, which recapitulates most of the main pathological features of the human disease, such as autophagy impairment and lipid droplet accumulation. Through lipidomic analysis, we aim to identify potential dysfunctions in lipid homeostasis.

Lipidomic profiling of *cln8*$^{-/-}$ and wild-type brains revealed significant dyslipidemia in mutant fish. Notably, the most dysregulated lipids include phospholipids, sulfatides, and ceramides.

• Abstracts e partecipazione a congressi e corsi: autori, titolo della presentazione, nome e date del congresso

- Sara Bernardi, Asahi Ogi, Federica Gemignani, Filippo M. Santorelli, Maria Marchese; Disentangling Batten disease through a zebrafish modelling: characterization of a new cln3-deficient zebrafish line; 4th Italian Zebrafish Meeting, Palermo 7-9 February 2024.
- Sara Bernardi; Asahi Ogi; Rosario Licitra, Giada Silvi; Serena Mero; Daniele Galatolo; Nicola Gammaldi; Stefano Doccini; Federica Gemignani; Gian Michele Ratto; Simona Rapposelli; Filippo M. Santorelli; Maria Marchese; Targeting autophagy in a novel zebrafish (Danio rerio) model on neuronal ceroid lipofuscinosis 8. 6th International Congress IRPES2024 – Pisa, December, 6th 2024
- Dual filaments regulation of muscle contraction, The molecular motor of muscle studied in vitro mechanics, Mechanical dysfunction in genetic-based cardiomyopathies: perspectives for the use of novel small molecules and biomimetic polymers, High-throughput sequencing: technologies and application in molecular medicine, Effects of SGLT-2 inhibitors in diseased myocardium.
- Corso di formazione in materia di protezione degli animali utilizzati a fini scientifici.
- The beta-adrenergic system in the retina: targeting beta adrenoceptors against retinal neovascular diseases, Prof. Massimo Dal Monte; The beta-adrenergic system in the retina: targeting beta adrenoceptors against retinal neovascular diseases, Dr. Rosario Amato (11-04-2024, Pisa).
- Prof. Reggiani Diversity and specialization of skeletal muscle fibers: an omics point of view; Prof.ssa Kronnie Circular RNA: the salt of the transcriptome
- Beyond the proteome: non-coding regulatory RNAs in cell pathophysiology. Part 1 and 2, Prof. Stefano Cagnin. (Siena, 20 e 21 maggio).
- Application of nano-agents in cancer and cerebral ischemia. Dott.Attilio Marino, Fondazione Stella Maris, Pisa.
- Organoids revolutionizing biomedicine through-miniature organ models (08/05/24). Webinar.
- Corso Embase. Webinar (Università di Firenze, 31/05/24)
- Corso PubMed. Webinar (Università di Firenze, 7/6/24)
- Corso diritto d'autore e pubblicare in accesso aperto (Università di firenze, 19/06/24)
- Corso trasversale "L'assicurazione della Qualità nell'Ateneo di Pisa e il ruolo dei dottorandi" (Università di Pisa, 20/9/24)
- Soft skill “Science and Society: continuity and change” (17/9/24)
- Soft skill “Come la terapia genica sta cambiando la storia di malattie ereditarie e tumorali” (27/9/24)

• Pubblicazioni scientifiche autori, titolo della pubblicazione, nome e numero della rivista, anno di pubblicazione

- Gammaldi, N., Doccini, S., Bernardi, S. et al. Dem-Aging: autophagy-related pathologies and the “two faces of dementia”. Neurogenetics 25, 39–46 (2024)
- Marchese M, Bernardi S, Ogi A, et al. Targeting autophagy impairment improves the phenotype of a novel CLN8 zebrafish model. Neurobiol Dis. 2024;197:106536.

- Santucci, L., Bernardi, S. et al. Impairment of glucose metabolism as a hallmark of progressive myoclonus epilepsies: focus on the neuronal ceroid lipofuscinoses. Frontiers in Cellular Neuroscience, 2024.

• Eventuali soggiorni in altri laboratori italiani o esteri

From August 14th to September 13th in Dr. Wendy E. Heywood's laboratory, Translational Mass Spectrometry Research group, Genetics and Genomic Medicine Research and Teaching Dept, UCL Institute of Child Health, London. For lipidomic profile analysis in Neuronal Ceroid Lipofuscinoses on Zebrafish samples.