Project title: "Whole Exome Sequencing and functional validation in zebrafish model to uncover new targets genes responsible for GnRH neuron development defects"

Tutor: Prof. Marco Bonomi (matricola n.17904)
Co-tutor: Dott.ssa Valeria Vezzoli (IRCCS Istituto Auxologico Italiano)

Mail: marco.bonomi@unimi.it; m.bonomi@auxologico.it

Congenital Hypogonadotropic Hypogonadism (CHH) is a rare disease due to a developmental failure of hypothalamic GnRH neurons.Early CHH diagnosIs is important to promptly establish treatments and ameliorate the individual reproductive competence, well-being and employment performance. CHH shows complex genetic and phenotypic heterogeneity. Approximately 50% of patients are still idiopathic, indicating that further regulatory genes remain to be identified.

Specific Aims:

1- Identify candidate genes in selected families by Whole Exome Sequencing. WES analysis will be performed on the genomic DNA of at least 5 probands and their relatives, carefully selected on the basis of their informative pedigrees.

2- ***Determine the biological relevance of novel CHH candidate genes in GnRH neuron development.***

To examine whether the identified candidate genes contribute to GnRH neuron development, we will combine:

2.1 Expression studies: For genes found to be expressed in primary and immortalized GnRH neurons and be conserved in zebrafish (zf), we will determine expression patterns with focus on key developmental stages of GnRH neurons;

2.2 Tissue culture models: we will use immortalized GnRH neuron cell lines to perform functional studies and define the natural role of the candidate genes. We will model the effect of identified variant *in silico* to predict which protein function may be compromised by using molecular modelling tools. Finally, we will study the impact of the mutation identified in our proband on protein function after site-directed mutagenesis in expression constructs.

2.3 Functional validation of novel candidate genes in zebrafish: To validate *in vitro* findings with *in vivo* models, we will study the impact of selected candidate gene knockout on GnRH neuron behaviour by genome editing *in vivo*, by using zf animal model and CRISPR/Cas9 technique.

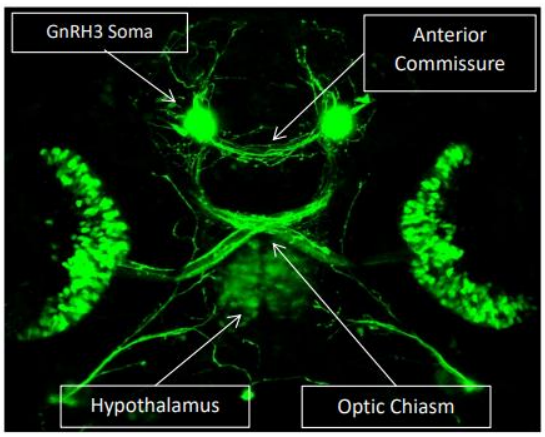
3-*Development of* GnRH *secreting neurons from patient Pluripotent Stem Cell.* Following a three-step protocol we aim to differentiate human pluripotent stem cells (hPSCs) from a patient with a seleceted CHH new candidate gene mutation, and their relatives, into GnRH-secreting neurons.

The described approach will provides an innovative translational tool for investigating the mechanisms of human puberty and validate the role of a new candidate gene in the CHH disease

"Whole Exome Sequencing and functional validation in zebrafish model to uncover new targets genes responsible for GnRH neuron development defects "

Whole Exome Sequencing

Zebrafish model



GnRH Neurons Development