Congenital hypogonadotropic hypogonadism (CHH) is a rare reproductive disorder characterized by deficiency or action failure of gonadotrophin-releasing hormone and delayed or arrested puberty. CHH shows complex genetic and phenotypic heterogeneity and approximately 50% of patients are still idiopathic, indicating that further regulatory genes remain to be identified.
In our cohort of CHH patients, we recently identified two heterozygous missense variants in the *WFS1* gene in two families. *WFS1* gene encodes for Wolframin, a ubiquitous protein involved in unfolded protein response, cell cycle progression, regulation of cellular calcium homeostasis and endoplasmic reticulum stress. Homozygous mutations of *WFS1* are responsible for "Wolfram Syndrome", a multisystem disorder characterized by visual dysfunctions, hearing loss, anosmia, diabetes mellitus or insipidus, psychiatric disorders and reduced fertility.
The identification of genetic variants in the *WFS1* gene in patients with CHH suggests a possible involvement of Wolframin during the various steps underlying the development and function of the hypothalamic-pituitary-gonadal (HPG)-axis.In this work, we took advantage of the zebrafish model to study the impact of *wfs1b* (orthologues of human *WFS1*) during the differentiation and migration of GnRH neurons. *In vitro* experiments on GN11, immortalized cell line established as a model for GnRH secreting neurons, were performed to verify the impact of the two allelic variants find by whole exome sequencing on the subcellular localization and expression of the WFS1 protein. Our analyses revealed that the mutated proteins have a lower expression in the ER and tend to form aggregates. In the zebrafish model we first evaluated the expression of *ws1b* mRNA in zebrafish embryos and adult tissues by RT-qPCR and WISH and we observed a nearly ubiquitous expression of *wfs1b and* in particular, the signal is highly detectable in brain and eyes. We next generated a knock down model for *wfs1b* through the co-injection of two splicing-blocking morpholinos designed on exon2/intron2 and exon7/intron7 of the pre-mRNA sequence. To evaluate the role of *wfs1b* on GnRH3 development we took advantage of *GnRH3*:EGFP transgenic line. All the *wfs1b*MOs showed a global disorganization of GnRH3 neuronal architecture that appeared mislocalized at the level of olfactory bulbs and anterior commissure. Moreover, most of them also exhibit a severe reduction of GnRH3 positive fibers in the optic chiasm and retina in comparison to controls. To evaluate the potential contribution of *wfs1b* during GnRH3 neurogenesis we analyzed by WISH experiments neuronal progenitors markers expressing the transcription factor *pax6* and intermediate neuronal precursors depicted by *ngn1* and *isl1a* expression. We observed a preserved expression of both *pax6* and *ngn1* in *wfs1b*MOs but interestingly, morphants embryos displayed differences in *isl1a* expression suggesting that the reduced expression of this marker may have a role in explaining GnRH3 migration defects. Due to the involvement of WFS1 in UPR mechanism we also performed RT-qPCR experiments in embryos at 48 hpf to investigate the expression of genes involved in the UPR and apoptosis pathways and our analysis revealed that levels of *bip, atf6,* and *chop* were significantly increased in *wfs1b*MOs suggesting that the knockdown of *wfs1b* activates UPR pathway and therefore, the apoptosis cascade.