

 <p><b>UniSR</b> Università Vita-Salute San Raffaele</p>	<p><b>APPLICATION TO ACT AS SUPERVISOR AND RESEARCH PROJECT PROPOSAL</b></p>	<p><b>MO 20-5</b> ed. 01 del 21/02/2025 PO 20 Page 4 of 8</p>
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**PROJECT**

**Supervisor:**

ALESSANDRA BOLETTA

**Title:**

REFINING THE MOLECULAR MECHANISM OF GLUTAMINE SENSING BY  
PRIMARY CILIA

**Curriculum:**

CELLULAR AND MOLECULAR BIOLOGY

Link to the personal page of the University or relevant hospital site website:

<https://research.hsr.it/en/divisions/genetics-and-cell-biology/cystic-kidney-disorders.html>

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

**Background/gap of knowledge**

Primary cilia are tiny solitary organelles present on the surface of most cells in our body. These are microtubule-based structures, non-motile and acting as cellular antennae on the surface of cells to capture various type of extracellular signals. These antennae are distributed in various different tissues, and accordingly their dysfunction can cause a variety of different manifestations. Inherited disorders caused by ciliary dysfunction are collectively called "ciliopathies". The most common of these disorders are renal inherited disorders called polycystic kidney disease. More rare forms of cystic disorders are also observed and the most frequent pediatric ciliopathy is called nephronophthisis. We have recently discovered a novel function for primary cilia. We have described that these organelles act as extracellular sensor of nutrient availability and convey this information to mitochondria to optimize their bioenergetic activity. In particular, cilia respond to glutamine, a non-essential-aminoacid which becomes conditionally essential during metabolic stress conditions as a primary source of carbon to be used in the TCA cycle in mitochondria. We have described that the enzyme responsible for this response is called Asparagine Synthetase (ASNS).

**Rationale and hypothesis**

Our data suggests that: i) cilia are essential for cells to sense and properly utilize glutamine for energy production; ii) cells lacking cilia have an impaired capability to respond to nutrient stress by utilizing glutamine; iii) the process is not dependent on the nutrient sensor mTORC1, but it depends on ASNS enzyme which enables glutamine utilization under stress conditions and which we have found located at the base of cilia. Here we hypothesize that the enzyme ASNS, being at cilia, is sensing and responding to the levels of extracellular glutamine. We will test the hypothesis in cellular systems, in murine kidneys and in a recently generated zebrafish model.

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**Objectives and specific aims**

The PhD student enrolled in this project will contribute to reach a better molecular definition of the process. In particular: i) (s)he will generate cells lacking the ASNS enzyme or expressing mutant forms of the enzyme to study its role in the ciliary response to glutamine; ii) characterize the trafficking of the ASNS enzyme tagged with GFP, RFP or mNeon; iii) complete the characterization of a zebrafish model recently generated by the lab and reconstitute it with human ASNS; iv) help validating the results of a cilia-specific proximity labelling proteomic study already conducted by the lab. Validations will be performed using cell lines, murine models and the novel zebrafish model.

**Expected outcomes**

We expect to unravel the molecular mechanism of the ciliary response to glutamine, in particular to define and refine the role of the metabolic enzyme ASNS, along with other possible players.

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

Upon completion of the project the fellow will be able to design and execute experiments independently. During the course of the project (s)he will learn how to perform Immunofluorescence and live imaging, western blots and qPCR analysis, cell cultures and tissues analysis (Immunohistochemistry), including analysis of tissues from mice and fish.

**References** (max. 15)

1. Anvarian Z, Mykytyn K, Mukhopadhyay S, Pedersen LB, Christensen ST. Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* 2019;15:199-219. doi: 10.1038/s41581-019-0116-9.
2. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat. Rev. Mol. Cell Biol.* 2017;18:533-547. doi: 10.1038/nrm.2017.60.
3. Steidl ME, Nigro EA, Nielsen AK, Pagliarini R, Cassina L, Lampis M, Podrini C, Chiaravalli M, Mannella V, Distefano G, Yang M, Aslanyan M, Musco G, Roepman R, Frezza C, Boletta A Primary cilia sense glutamine availability and respond via asparagine synthetase. *5:385-397*, 2023. doi: 10.1038/s42255-023-00754-6.