NEXT GENERATION SEQUENCING (NGS) APPROACH TO DEEPEN INTO PHOX2B MUTATIONS MEDIATED PATHOGENESIS AND TO IDENTIFY DRUGS ABLE TO RESCUE CELLULAR AND MOLECULAR DEFECTS IN CCHS

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Isabella Ceccherini (center) and team

Hypoxia and hypercapnia arise when falling asleep, unless autonomic respiration takes place. In CCHS patients the ventilatory response to such a physiological challenge is abnormal. For this reason, CCHS is a lifelong disease that does not resolve spontaneously, and clinicians and families manage patients only by chronic ventilatory supports. The PHOX2B gene encodes a protein crucial for the development of the autonomous nervous system. One PHOX2B mutation, either inherited by one parent or arising de novo in the patient, is enough to cause CCHS. In particular, the expansion of a poly-alanine (polyAla) stretch from +4Ala residues to +13Ala residues is the most frequent PHOX2B mutation, accounting for 85% of cases. In addition, rarer so-called missense, nonsense, and frameshift mutations can also occur in CCHS patients, all together accounting for 15% of cases.

CCHS is an orphan disease as it has not been "adopted" yet by the pharmaceutical industry to develop and market new medications. *In vitro* studies and a serendipitous observation in patients have provided preliminary evidence that the molecular consequences of PHOX2B mutations, particularly polyAla expansions, can be reversed. This is the case of the activation of the so-called heat shock response by the drug geldanamycin (GA), its analogue 17-AAG and the natural compound curcumin that, in cell model systems of CCHS, have been observed to exert beneficial effects. Desogestrel has also been fortuitously observed to recover ventilatory response to hypercapnia in two CCHS patients taking this very potent progestin as a contraceptive treatment. However, in no case, at the best of our knowledge, these drugs have entered into a pharmacological approach to CCHS treatment *in vivo*.

One of the main obstacles preventing the development of a pharmacological strategy effective in counteracting the damaging effects of PHOX2B mutations in CCHS is the limited knowledge we have gained so far about the patho-physiology of PHOX2B: the genes it regulates, the companion protein factors it interacts with, the time when and the place where its action is indispensable, the pathways it is involved in, the gene networks it gives rise to are poorly known or still lacking information.

Indeed, translational medicine programs, aimed at deepening into the molecular details of PHOX2B mutations mediated CCHS pathogenesis, are essential to develop pharmacological therapies that would bring an enormous improvement in the quality of life of CCHS patients and their families.

Therefore, in the present pilot study, 1) genes and pathways involved in PHOX2B mutations mediated pathogenesis will be identified by assessing the gene expression profile of cell lines bearing different PHOX2B mutation; 2) these results will be used, applying proper statistical approaches, to rank genes and pathways resulting to drive the most deleterious effects of PHOX2B mutations in CCHS cell models: the top ones will represent highly desired drug targets; 3) drugs that may have a beneficial effect in CCHS will be predicted starting from above differential gene expression data and taking advantage of available online tools *(t*he Ingenuity Pathway Analysis (IPA), the Conne*ctivity* Map (cMap), and the Mode of Action by NeTwoRk Analysis (MANTRA)) that do examine the connections among diseases, genes and drugs.

During successive steps of the research, drugs thus identified as potentially beneficial will be validated *in vitro* and eventually assessed in clinical trials.