The N-terminal domain aggregation and alanine expansion associated with Congenital Central Hypoventilation Syndrome impairs dimerization and homeodomain functions of PHOX2B: possible targets for pharmacological intervention

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Congenital Central Hypoventilation Syndrome (CCHS) is a very rare neonatal neurological disorder characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control: affected children hypoventilate during sleep, with possible very severe neurological damages.
Frameshift mutations (5%) and poly-alanine triplet expansions (95%) have been detected in the coding region of the homeobox gene PHOX2B in about 90% of CCHS patients. A correlation between length of the expansion and severity of the respiratory phenotype has been reported. So far no pharmacological treatments are available for CCHS, and patients rely on ventilator support such as tracheotomy, nasal mask, or diaphragm pacing by phrenic nerve stimulation. Recently it has been shown that the progestin Desogestrel ameliorated the chemo-sensitivity response in two CCHS female patients. Our recent data show that Desogestrel augments the activity of the wild-type as well as mutant proteins by a not yet identified molecular mechanism. Since some of the mutations alter the sub-cellular localization, the DNA-binding affinity and the transcriptional activity of the protein, and that mutated PHOX2B proteins can interfere with the activity of the wild-type protein by sequestering it into aggregates, one crucial question concerns the identification of the functional domains of the protein, the role of the poly-alanine tract and the effects of its expansion on the general architecture and function of the protein, in order to characterize the mechanism underlying the Desogestrel effect. We have performed a deletion analysis of PHOX2B and identified two nuclear localization signals in the homeodomain, both required for the complete import of the protein in the nucleus, corresponding to residues necessary for the binding to DNA, and partially blocked by the expanded poly-alanine tract. By using mammalian two-hybrid system we have also demonstrated that PHOX2B can form homo-dimers but does not heterodimerize with mutated proteins, and exclude the involvement of the polyalanine tract in dimer formation. Moreover we show that the N-terminal domain has strong tendency to aggregate. These results provide novel insight into the role of the alanine tract and of other domains for the protein activity and into the effect of the alanine tract expansion on PHOX2B folding, contributing to the range of phenotypes and pathogenesis in CCHS patients. The involvement of the different domain in the response to Desogestrel will be further investigated.