**Effects of PHOX2B mutations and ALK expression on neuroblastoma pathogenesis**

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**Abstract**

Molecular analysis in congenital central hypoventilation syndrome (CCHS) has revealed the presence of four types of mutations in the coding region of *PHOX2B*: frameshift (FS), missense (MS), nonsense and polyalanine triplet expansion mutations.

The *PHOX2B* gene encodes a transcription factor which is involved in the specification of the noradrenergic phenotype during the development and differentiation of neural crest derivatives.

In a cohort of 100 CCHS patients screened so far frameshift mutations have been detected in 10% of samples, missense mutations in 3,2% and nonsense mutation in 1%, in addition to the most frequent mutation, an in-frame duplication leading to expansions from +5 to +13 alanine residues within a 20 alanine tract in the exon 3.

In the past years, we have characterized molecular mechanisms of polyAla expansions and of the frameshift mutations c.614–618delC, c.862–866insG, c.721–758del38nt and c.930insG. In particular, we have already reported that all mutations do impair the PHOX2B transcriptional activity by different processes: while polyAla expansions severely reduce the PHOX2B transcriptional activity because of polyAla length dependent cytoplasmic retention of the transcription factor, such mechanism was excluded for FS mutation, and the role of PHOX2B interactome and/or downstream targets has been suggested among their pathogenic effects.

Differently from polyAla expansions, MS and FS mutations are associated with a high risk for CCHS patients to develop neuroblastoma (NB), the most frequent neural crest cells derived childhood tumor. Germline and somatic mutations of both *PHOX2B* and, more recently, of the Anaplastic Lymphoma Kinase (*ALK)* gene have been detected in association with both hereditary and sporadic neuroblastoma (NB). Recently, we have shown a close relationship between the two genesdemonstrating that both are overexpressed in NB and that PHOX2B drives *ALK* gene transcription by directly binding its promoter.

To better deepen into the relationship between PHOX2B mutations and ALK expression in the pathogenesis of NB, we have sought to investigate the role of polyAla and nonPolyAla mutations in transactivating *ALK* promoter, in particular testing novel frameshift, missense and nonsense mutations recently detected in our CCHS cases. Preliminary data of this study show an impaired transactivation of ALK promoter mediated by polyAla (+13) expansion compared to wild type protein. Among the many frameshift mutations detected so far, the effects of c.614-618delC, an aberrant shift of the wilt type reading frame resulting in a truncated protein of 307 amino acids, have also been tested, observing a remarkable decreased transactivation of *ALK* promoter.

As PHOX2B wt has been observed to increase ALK expression, we are going to evaluate the effects of additional NB associated *PHOX2B* mutations and PolyAla expansions to disclose molecular mechanisms underlying NB associated- or isolated- CCHS .