**MOLECULAR DIAGNOSIS OF CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS) IN SPAIN**

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*Introduction*: CCHS is an autosomal dominant disorder caused in >90% of patients by heterozygous in frame duplications of the second polyAla-rich repeat (PARM, Poly-Alanine Repeat-Mutations), located in exon 3 of *PHOX2B*. Missense, nonsense and frameshift *PHOX2B* mutations (NPARM, Non-Poly-Alanine-Repeat-Mutations) explain most of the remaining cases. There is a genotype/phenotype correlation between the length of the PARMs and ventilatory aid dependence as well as between the mutation type and the severity of the phenotype (NPARMs>PARMs). Hence, the molecular analysis of *PHOX2B* is essential to confirm the clinical diagnosis of CCHS and to anticipate the severity of CCHS phenotype.

*Aim:* To establish a reference laboratory for the genetic diagnosis of CCHS in Spain by: 1) Organizing a central registry of patients with a clinical diagnosis of CCHS; 2) Collection of DNA samples from CCHS affected/suspected patients and relatives, after obtaining informed consent. 3) Implementing highly sensitive molecular diagnostic protocols for the mutation screening of *PHOX2B,* based on High Resolution Melting analysis (HRM) and DNA sequencing.

*Subjects:*  A total of 35 patients, recruited from 16 different Spanish Hospitals, and 41 patient relatives, have been collected and analysed to date. 21 had a clinical diagnosis of CCHS and 14 were suspected CCHS newborns.

*Methods*: DNA was extracted from blood lymphocytes. The coding sequences, intron/exon boundaries and known regulatory regions of *PHOX2B* were amplified by PCR and screened for mutations by the combination of HRM (LightScanner HR 96) and DNA sequencing of all detected variants.

*Results:* HRM detected a *PHOX2B* mutation in 24/35 patients (18/21 with a clinical CCHS diagnosis and 6/14 were suspected CCHS newborns). 21/24 (87.5%) were PARMs (20/27, n=8; 20/26, n=7; 20/25, n=3; 20/33, n=3) and 3/24 (12.5%) NPARMs: p.R141Q, c.618dup, and c.691\_698del. One of the three patients with the 20/33 PARM required continuous ventilatory support until the age of 7 years; two of the 7 patients with 20/27 PARM required a cardiac pacemaker implantation; five patients presented with Hirschprung disease (2 with PARM 20/33; 1 with 20/27, and 2 with NPARMs). None of the identified mutations was detected in the unaffected tested relatives. The estimated sensitivity of the HRM assay for the detection of both *PHOX2B* PARMs and NPARMs was >99% with a specificity of >98%.

*Conclusions:* 1) We have established a reference laboratory for the genetic diagnosis of CCHS in Spain; 2) HRM is a quick, highly sensitive screening technique for the detection of *PHOX2B* mutations in the diagnosis of CCHS.3) *PHOX2B* mutation spectrum in Spanish CCHS patients is similar to that previously described in other countries; 4) As previously reported, CCHS phenotype severity correlated with both the mutation type (NPARMs>PARMs) and the length of the PARMs.