Title: Paired-like Homeobox Gene PHOX2B Non-Polyalanine Repeat Expansion Mutations: Genotype-phenotype Correlation In Congenital Central Hypoventilation Syndrome And Later Onset-CCHS

Author Block: S. Hockney1 , A. Zhou1 , G. Niewijk1 , C. Rand1 , P. Reineke2 , V. Speare2 , E. Berry-Kravis3 , L. Zhou3 , L. Jennings4 , M. Yu4 , I. Ceccherini5 , K. Yap4 , D. E. WeeseMayer6

Insitution: 1Department of Pediatrics--Division of Autonomic Medicine, Ann & Robert H Lurie Children's Hospital of Chicago and Stanley Manne Children's Research Institute, CHICAGO, IL, United States, 2Ambry Genetics, Aliso Viejo, CA, United States, 3Departments of Pediatrics, Neurological Sciences and Biochemistry, Rush University Medical Center, CHICAGO, IL, United States, 4Department of Pathology, Ann & Robert H Lurie Children's Hospital of Chicago, CHICAGO, IL, United States, 5Gaslini Instituto, Genoa, Italy, 6Department of Pediatrics--Division of Autonomic Medicine, Ann & Robert H Lurie Children's Hospital of Chicago and Stanley Manne Children's Research Institute and Northwestern University Feinberg School of Medicine, CHICAGO, IL, United States.

Rationale: PHOX2B is the disease-defining gene for Congenital Central Hypoventilation Syndrome (CCHS) and Later-Onset CCHS (LO-CCHS). We previously described a PHOX2B genotype-CCHS phenotype relationship for heterozygous polyalanine repeat expansion mutations (PARMs; causing ~90% of CCHS), allowing risk assessment for phenotypic features. Phenotypes associated with the rarer non-PARMs (NPARMs; causing ~10% of CCHS) remain unclear. We developed a collaboration to advance understanding of NPARMs through compilation of new and previously published NPARMs and commonly related phenotypes, including CCHS/LO-CCHS diagnosis, Hirschsprung disease, neural crest tumors, and artificial ventilatory needs.

Methods: NPARM information was requested from 18 clinical academic, 20 commercial, and 11 research laboratories. Obtained information was paired with peer-reviewed publications reporting PHOX2B NPARM cases. Phenotypic information was collected from PHOX2B testing order forms, published manuscripts, and registry sources, but noted to be incomplete for a subset of reported NPARMs. Categorical data were analyzed with Fisher's exact test and Bonferroni correction.

Results: 295 NPARM variants were identified (136 unique; Table 1), including 75 previously unreported unique variants, and categorized by variant type and exon location, with available details regarding phenotype. Findings demonstrate that CCHS-causing PHOX2B frameshift, missense, nonsense, and in-frame variants are typically found in different exons (p<0.001), with frameshift and in-frame variants largely in exon 3, missense variants largely in exon 2 and nonsense variants largely in exon 1. Among all variant types, there are significant differences between variants for the following phenotypic features: LO-CCHS (p=0.002), Hirschsprung disease (p<0.001) and artificial ventilatory needs of continuous ventilation vs. sleep only (p=0.002). Based on variant location in the PHOX2B gene, there are significant differences between location and occurrence of presentation after one month of age (LO-CCHS; p<0.001).

Conclusions: This study presents the largest cohort of PHOX2B NPARMs and associated phenotype data to date, increasing knowledge on type and location of CCHS-associated NPARMs and associated phenotypes. Collating NPARMs and associated phenotypic manifestations represents a first step toward developing personalized, anticipatory clinical management guidelines based on mutation type and

location, and should also guide future investigation of pathogenic mechanisms. Results clearly demonstrate a high frequency of later-onset presentation with some mutation locations and types, highlighting the need for consideration of thorough PHOX2B testing in patients presenting with non-traditional CCHS symptoms (such as presentation outside the newborn period). Some phenotypic frequencies are likely underestimated due to PHOX2B analysis preceding diagnosis of full clinical features. Follow-up is essential to characterize the full phenotype with advancing age.