Of Mice and Babies: PHOX2B and Obstructive Apneas in Congenital Central Hypoventilation Syndrome

From birth to death, breathing and upper airway function are precisely regulated by a neural network located in the brainstem and pons that generates respiratory rhythm and pattern in a sleep–wake state-dependent manner (1). Central respiratory chemoreceptors sense very small changes in brain P_{CO_2} and activate the respiratory centers maintaining the stability of CO_2 (2). The retrotrapezoid nucleus (RTN) resides at the ventral medullary surface and includes a well-defined subset of CO_2-sensitive neurons characterized by the expression of *Phox2b* (paired-like homeobox gene 2b), Vglut2 (vesicular glutamate transporter 2), Nmb (neuromedin B), TASK-2 (two-pore-domain potassium channel 2), and GPR4 (G-protein–coupled receptor 4) (3). Failure to detect P_{CO_2} fluctuations by RTN neurons and other central chemoreceptors leads to severe diseases such as congenital central hypoventilation syndrome (CCHS), which result in hypercapnic respiratory failure. The CO_2 chemoreflex plays an important role in defense mechanisms against obstructive sleep apnea by activating hypoglossal motoneurons and the main pharyngeal dilator, the genioglossus muscle (4).

CCHS is a rare, autosomal dominant, and life-threatening disorder first described in 1970 by Mellins and colleagues, who suggested that "injury to medullary chemoreceptors is responsible for alveolar hypoventilation in this syndrome" (5). CCHS is characterized by an impaired ventilatory response to CO_2, mainly during sleep, hypoxemia, facial dysmorphology, cardiac asystoles, Hirschsprung disease, and neuroblastoma (6). CCHS results from the mutation of *Phox2b* on chromosome 4p12, which is essential for the development of the respiratory control centers (7). The incidence of CCHS is estimated at 1/148,000 live births (8). There is no approved therapy for CCHS and its devastating complications. Like humans with CCHS, transgenic mice with *Phox2b* mutation develop hypoventilation during sleep, central sleep apneas, and decreased hypercapnic ventilatory response (7, 9). However, the effect of *Phox2b* deficiency on upper airway obstruction during sleep is unknown. Experiments in transgenic mice allow us to address two important questions: *1*) Can a mouse model offer an insight into the mechanisms of different types of apneas in neonates? *2*) Does *Phox2b* deficiency in CO_2-sensing neurons predispose to obstructive apneas?

Investigators previously developed and validated plethysmographic methods for monitoring airflow and respiratory effort, which allowed the detection of upper airway obstruction during sleep in adult mice (10, 11). However, technology allowing us to differentiate between obstructive and central events in newborn animals did not previously exist (12).

In this issue of the *Journal*, Madani and colleagues (pp. 1200–1210) report that the $Phox2b^{27Ala/+}$ mutation predisposes neonatal mice to CCHS, manifested by central as well as obstructive and mixed apneas caused by hypoglossal dysgenesis (13). Madani and colleagues generated two mouse strains that carry the most common of the CCHS-causing mutations, a +7 alanine expansion of the 20-residue polyAla tract (the $Phox2b^{27Ala}$ allele), by a knock-in approach. The first strain carried the $Phox2b^{27Ala/+}$ transgene globally, whereas in the second strain, $Phox2b^{27Alacond/+}$ mice, the mutation was expressed exclusively in the RTN neurons. The investigators developed the state-of-the-art technology to detect obstructive events. They performed three-dimensional (3D) scans of newborn mice and created custom-made pneumotachograph/face masks using 3D printing (Figure 1). This interesting approach was combined with a laser profilometer pointing radially at the lateral abdominal wall to detect abdominal effort, which allowed the differentiation of central, obstructive, and mixed events. *In vivo* data were complemented by morphometry of the hypoglossal nucleus and electrophysiology in brain slices. The authors provided convincing evidence that newborn mice with the global $Phox2b^{27Ala/+}$ mutation have markedly increased obstructive apneas in addition to central and mixed events. In contrast, $Phox2b^{27Alacond/+}$ deficiency localized to the RTN did not induce obstructive events. Morphometry showed that global $Phox2b^{27Ala/+}$ deficiency led to hypoplasia of the hypoglossal nucleus, and an electrophysiology study demonstrated that the mutation was associated with a decreased burst frequency of the hypoglossal motoneurons and discoordination of hypoglossal and phrenic nerve activity.

The hypoglossal nerve (12N) maintains upper airway muscle tone and pharyngeal patency by modulating activity of the tongue protruder genioglossus and retractors styloglossus and hyoglossus (14). The major finding of the paper is that global, but not RTN-specific, *Phox2b* mutation leads to hypoplasia and dysfunction of hypoglossal motoneurons as a likely cause of obstructive apneas in neonate mice.

The study has several limitations. First, mechanisms of the *Phox2b* defect in the hypoglossal motoneurons and upper airway function remain unclear. The investigators attributed the deficit to primary dysfunction of hypoglossal motoneurons, but the cause of this dysfunction is not clear, besides that it occurs outside of the RTN. In fact, hypoglossal motoneurons do not express PHOX2B, but this protein is found in the other brain regions, such as the nucleus tractus solitarii, which contributes to the hypercapnic ventilatory response (15). Whether or not PHOX2b-expressing neurons in the nucleus tractus solitarii are involved in obstructive apnea in CCHS is a matter deserving further investigation (Figure 1). Second, global *Phox2b* deficiency appeared to have a much more impressive impact on the apnea duration compared with the RTN-specific defect, which suggests severe impairment of control of breathing. Obstructive and mixed apnea in these animals are much longer than central events. Given technical challenges to differentiate between obstructive and mixed events, it opens a possibility that the airway may simply close at the end of a very long central event, as it is described in human

V.Y.P. is supported by NIH grants R01 HL128970, R01 HL133100, R01 HL13892, R61HL156240, and U18DA052301.

Originally Published in Press as DOI: 10.1164/rccm.202108-1989ED on October 11, 2021

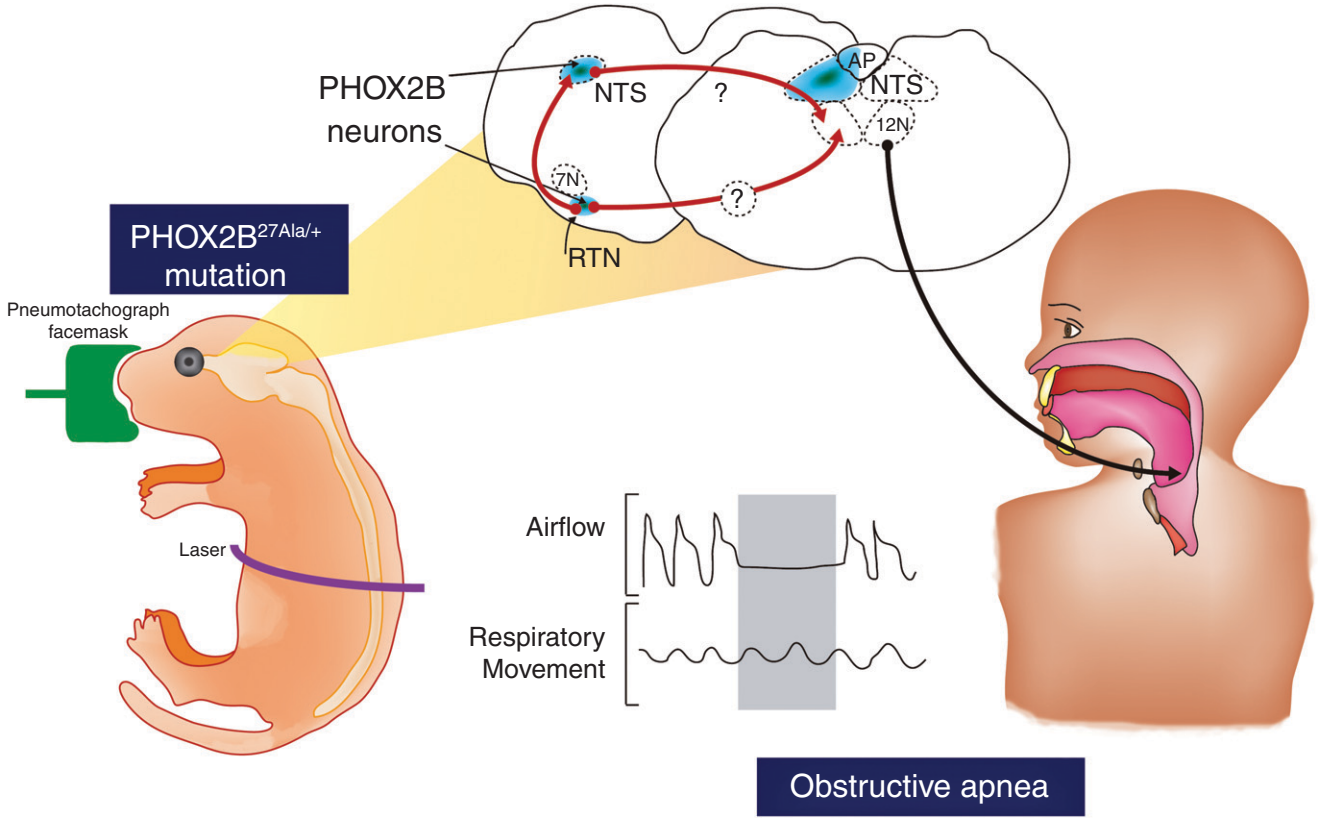

Figure 1. Summary of the main findings of Madani and colleagues. The investigators generated a mouse model of congenital central hypoventilation syndrome and validated the custom-made pneumotachograph/facemasks combined with a laser profilometer to detect abdominal effort. Putative monosynaptic or polysynaptic projections from *Phox2b* neurons in the NTS or RTN to the 12N may be necessary for upper airway patency during sleep. 7N = facial nucleus; 12N = hypoglossal nucleus; AP = area postrema; NTS = nucleus tractus solitarii; PHOX2B = paired-like homeobox gene; RTN = retrotrapezoid nucleus.

obstructive sleep apnea (16). Third, although *in vivo* data suggest the lack of RTN *Phox2b* involvement in hypoglossal motoneuron dysfunction, future mechanistic studies using morphometry and electrophysiology in mice with *Phox2b* knockout specific to RTN are necessary to test this hypothesis.

In conclusion, despite its limitations, the study by Madani and colleagues represents a significant advancement in the field because it raises the possibility that *Phox2b* mutation may lead to obstructive apneas along with centrals. This seminal observation together with the elegant animal model developed in this study may allow further investigation of the individual role of brain regions in sleep-disordered breathing beyond CCHS. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Mateus Ramos Amorim, Ph.D.
Department of Medicine
Johns Hopkins University School of Medicine
Baltimore, Maryland

Raouf Amin, M.D.
Division of Pulmonary Medicine
Cincinnati Children's Hospital Medical Center
Cincinnati, Ohio

and

Department of Pediatrics
University of Cincinnati College of Medicine
Cincinnati, Ohio

Vsevolod Y. Polotsky, M.D., Ph.D.
Department of Medicine
Johns Hopkins University School of Medicine
Baltimore, Maryland

References

1. Smith JC, Abdala APL, Koizumi H, Rybak IA, Paton JFR. Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. *J Neurophysiol* 2007;98: 3370–3387.
2. Feldman JL, Mitchell GS, Nattie EE. Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* 2003;26:239–266.
3. Guyenet PG, Stornetta RL, Souza GMPR, Abbott SBG, Shi Y, Bayliss DA. The retrotrapezoid nucleus: central chemoreceptor and regulator of breathing automaticity. *Trends Neurosci* 2019;42:807–824.
4. Pillar G, Malhotra A, Fogel RB, Beauregard J, Slamowitz DI, Shea SA, *et al.* Upper airway muscle responsiveness to rising PCO(2) during NREM sleep. *J Appl Physiol (1985)* 2000;89:1275–1282.
5. Mellins RB, Balfour HH Jr, Turino GM, Winters RW. Failure of automatic control of ventilation (Ondine's curse). Report of an infant born with this

syndrome and review of the literature. *Medicine (Baltimore)* 1970;49: 487–504.
6. Weese-Mayer DE, Berry-Kravis EM, Ceccherini I, Keens TG, Loghmanee DA, Trang H; ATS Congenital Central Hypoventilation Syndrome Subcommittee. An official ATS clinical policy statement: Congenital central hypoventilation syndrome: genetic basis, diagnosis, and management. *Am J Respir Crit Care Med* 2010;181:626–644.
7. Amiel J, Laudier B, Attié-Bitach T, Trang H, de Pontual L, Gener B, *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat Genet* 2003;33:459–461.
8. Shimokaze T, Sasaki A, Meguro T, Hasegawa H, Hiraku Y, Yoshikawa T, *et al.* Genotype-phenotype relationship in Japanese patients with congenital central hypoventilation syndrome. *J Hum Genet* 2015;60: 473–477.
9. Dubreuil V, Ramanantsoa N, Trochet D, Vaubourg V, Amiel J, Gallego J, *et al.* A human mutation in Phox2b causes lack of CO2 chemosensitivity, fatal central apnea, and specific loss of parafacial neurons. *Proc Natl Acad Sci USA* 2008;105:1067–1072.
10. Hernandez AB, Kirkness JP, Smith PL, Schneider H, Polotsky M, Richardson RA, *et al.* Novel whole body plethysmography system for the continuous characterization of sleep and breathing in a mouse. *J Appl Physiol (1985)* 2012;112:671–680.
11. Fleury Curado T, Pho H, Berger S, Caballero-Eraso C, Shin M-K, Sennes LU, *et al.* Sleep-disordered breathing in C57BL/6J mice with diet-induced obesity. *Sleep (Basel)* 2018;41:zsy089.
12. Cai J, Tuong CM, Gozal D. A neonatal mouse model of intermittent hypoxia associated with features of apnea in premature infants. *Respir Physiol Neurobiol* 2011;178:210–217.
13. Madani A, Pitollat G, Sizun E, Cardoit L, Ringot M, Bourgeois T, *et al.* Obstructive apneas in a mouse model of congenital central hypoventilation syndrome. *Am J Respir Crit Care Med* 2021;204:1200–1210.
14. Cori JM, O'Donoghue FJ, Jordan AS. Sleeping tongue: current perspectives of genioglossus control in healthy individuals and patients with obstructive sleep apnea. *Nat Sci Sleep* 2018;10:169–179.
15. Fu C, Shi L, Wei Z, Yu H, Hao Y, Tian Y, *et al.* Activation of Phox2b-expressing neurons in the nucleus tractus solitarii drives breathing in mice. *J Neurosci* 2019;39:2837–2846.
16. Badr MS, Toiber F, Skatrud JB, Dempsey J. Pharyngeal narrowing/occlusion during central sleep apnea. *J Appl Physiol (1985)* 1995;78: 1806–1815.

Characterization of Air Pollution Exposures as Risk Factors for Tuberculosis Infection

Tuberculosis is a leading cause of death owing to infectious disease worldwide, accounting for 1.4 million deaths of the 10 million persons with active tuberculosis disease in 2019 (1). Although improvements in the diagnosis and management of tuberculosis have led to declines in mortality over the past two decades, annual incidence remains unchanged. With the rapid growth in the world's population, which currently includes approximately 1.7 billion persons with prevalent latent tuberculosis infection (2), a higher absolute number of incident tuberculosis cases progressing from latent infection threatens to offset gains from improved case detection and treatment success. At the first-ever United Nations High Level Meeting on Tuberculosis in 2018, new commitments were made to scaling up prevention measures. However, low rates of uptake and completion of tuberculosis preventive therapy and lack of highly effective vaccines highlight the need for new public health approaches to tuberculosis prevention (1).

One area of tuberculosis epidemiology that offers opportunities for improving prevention is enhancing our understanding of how exposure to environmental air pollutants may influence the risks of transmission and progression from latent infection to active disease (3). For example, occupational exposure to dust and smoke, including silica, are well-recognized risk factors for active tuberculosis disease (4, 5). Second-hand exposure to tobacco smoke is also associated with a moderately higher risk of latent tuberculosis infection and a dose-dependent risk of active tuberculosis disease, especially among children (6). Exposure to solid fuel smoke and kerosene for heating and cooking are other potential factors that may increase the risk of active tuberculosis disease; however, results from epidemiological studies remain mixed (7–10), and better studies are needed to understand if these are important risk factors. Even less is known about ambient air pollution. At least one analysis of data from Beijing and Hong Kong has shown an association between seasonal concentrations of particulate matter $\leqslant$2.5 μm in aerodynamic diameter ($PM_{2.5}$) and active tuberculosis case notifications (11).

In this issue of the *Journal*, Blount and colleagues (pp. 1211–1221) present the results of a cross-sectional study examining associations between multiple urban environmental exposures and latent tuberculosis infection among 109 child household contacts of 72 patients with active tuberculosis in Hanoi, Vietnam (12). Key indoor air pollution exposure variables were assessed by questionnaire and were complemented by measurement of personal exposures to $PM_{2.5}$ over 48–72 hours. The authors constructed a multiple variable logistic regression model to explore the associations between these exposures and the outcome of latent tuberculosis infection, as defined by a positive tuberculin skin test.

This study makes some important observations. First, it confirms findings of a positive association between secondhand tobacco smoke exposure and latent tuberculosis infection. Second, the authors found that living on the ground floor was associated with a higher risk of latent tuberculosis infection when compared with floors above ground level. The authors conclude that indoor air pollution

Originally Published in Press as DOI: 10.1164/rccm.202107-1795ED on August 30, 2021