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SCIENTIFIC INVESTIGATIONS

Variable phenotypes in congenital central hypoventilation syndrome with *PHOX2B* nonpolyalanine repeat mutations

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Study Objectives: Congenital central hypoventilation syndrome (CCHS) is a rare disorder affecting the autonomic nervous system that is caused by variants in the paired-like homeobox 2B (*PHOX2B*) gene. About 10% of patients with CCHS have nonpolyalanine repeat mutations (NPARM) that are associated with severe phenotypes requiring continuous assisted ventilation, Hirschsprung's disease, and increased neural crest tumor risk. However, some patients with NPARM have milder phenotypes. Our objective was to describe the phenotypes in patients with CCHS *PHOX2B* NPARM.

Methods: Retrospective case series of patients with CCHS *PHOX2B* NPARM was conducted at 2 children's hospitals to evaluate their phenotypes.

Results: We identified 8 patients with CCHS *PHOX2B* NPARM aged 3–31 years. Seven patients were diagnosed in infancy and 1 patient at 2 years of age. All patients presented with respiratory depression in the first 2 months of life. Only 1 patient was identified with a severe phenotype requiring continuous assisted ventilation, Hirschsprung's disease, and a neural crest tumor, which was resected. Five patients required positive pressure ventilation via tracheostomy only during sleep and 2 patients required oxygen only during sleep. Four patients had Hirschsprung's disease and 1 patient had a cardiac pacemaker due to a bradyarrhythmia. None of the patients had echocardiographic abnormalities.

Conclusions: Patients with CCHS *PHOX2B* NPARM can have variable phenotypes, emphasizing the importance of implementing a plan of care that is individualized for each patient. The type of NPARM and their respective location on the *PHOX2B* gene may play a critical role in the severity of phenotypes displayed by each patient.

Keywords: congenital central hypoventilation syndrome, CCHS, PHOX2B, NPARM

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Nonpolyalanine repeat mutations of the paired-like homeobox 2B gene in patients with congenital central hypoventilation syndrome are generally associated with severe phenotypes requiring continuous assisted ventilation, Hirschsprung's disease, and neural crest tumors. Because patients with relatively milder or variable phenotypes have been reported, we sought to determine the clinical features in our cohort of patients with nonpolyalanine repeat mutations.

Study Impact: This study shows that patients with congenital central hypoventilation syndrome and nonpolyalanine repeat mutations can have variable phenotypes. It demonstrates the importance of regular surveillance studies to determine each patient's phenotype and individualized care.

INTRODUCTION

Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder caused by variants in the paired-like homeobox 2B (*PHOX2B*) gene.[1] Most patients with CCHS present in the newborn period with apnea, hypoxemia, and hypoventilation requiring assisted ventilation. Due to impaired central regulation of breathing, individuals with CCHS require lifelong assisted ventilation that can be delivered by positive pressure ventilation via tracheostomy (PPV-T), noninvasive positive pressure ventilation (NPPV), and/or diaphragm pacing.[1,2] The spectrum of ventilatory abnormalities ranges between sleep-disordered breathing, central sleep apnea, and hypoventilation during sleep needing assisted ventilation to hypoventilation even during wakefulness requiring continuous assisted ventilation.[1,2] Because CCHS is a generalized disorder of the autonomic nervous system (ANS), affected patients may manifest features of ANS dysfunction, such as bradycardia, cardiac arrhythmia that may require implantation of a cardiac pacemaker, Hirschsprung's disease (HD), and tumors of neural crest origin (NCT) such as neuroblastoma, ganglioneuroma, and ganglioneuroblastoma.[1,3]

The *PHOX2B* gene is located on chromosome 4p12 and encodes a transcription factor that is important for the development of the ANS. The *PHOX2B* gene normally contains a repeat sequence of 20 alanines in exon 3. The majority (90%) of patients with CCHS have increased polyalanine repeats in exon 3 of the *PHOX2B* gene resulting in polyalanine repeat expansion mutations (PARM). Approximately 10% of patients are heterozygous for nonpolyalanine repeat mutations (NPARM) in the *PHOX2B* gene that include frameshift, nonsense, and missense mutations, whereas less than 1% have *PHOX2B* exon or whole gene deletion.[1,4,5] The majority of NPARM are reported to cause severe phenotypes with need for continuous assisted ventilation, Hirschsprung's disease, and increased NCT risk.[6] However, there are

Table 1—Patient characteristics and *PHOX2B* NPARM genotype.

Patient	Age (y), Sex	*PHOX2B* Gene Variant (NM_003924.3)	Clinical Presentation	Age at Diagnosis	Parental *PHOX2B* Origin
1	5, F	c.245C>T (p.Pro82Leu) in exon 2	Apnea, respiratory distress, bradycardia at birth	2 months	Inherited from asymptomatic mother
2	31, F	c.245C>T (p.Pro82Leu) in exon 2	Apnea, cardiorespiratory arrest at 2 weeks age	4 weeks	16.7% allele frequency (mosaicism) in mother
3	11, F	c.945A>G (p.*315Trpext*41) in exon 3	Cyanosis at birth, HD	9 months	N/A
4	27, M	c.428A>G (p.Gln143Arg) in exon 2	Apnea, cyanosis at birth, HD	5 weeks	De novo
5	3, M	c.547G>T (p.Glu183Ter) in exon 3	ALTE at 7 weeks of age, OSA, recurrent apneas	2 years	N/A
6	7, F	c.432_433 delGTinsTC (p.Trp145Arg) in exon 3	HD at birth, apnea, and hypoxemia at 6 weeks age	2 months	N/A
7	3, M	c.448C>T (p.Arg150Cys) in exon 3	Apnea at birth, respiratory failure at 2 months with viral respiratory infection	3 months	N/A
8	3, F	c.242-1G>A in intron 1 splice acceptor	Hypoxemia, hypotonia, feeding problems at birth, and OSA	9 months	De novo

ALTE = apparent life-threatening event, F = female, HD = Hirschsprung's disease, M = male, N/A = not available, NPARM = nonpolyalanine repeat mutation, OSA = obstructive sleep apnea, *PHOX2B* = paired-like homeobox 2B.

several case studies of patients with NPARM and relatively milder phenotypes.[7–13] Respiratory manifestations in these cases include central apneas with hypoxemia,[9,14] central sleep apnea,[10,15] and requirement of assisted ventilation only during sleep.[7,8,11,13,16–18] Moreover, some individuals with NPARM may be asymptomatic and are only identified by genetic studies due to an affected family member.[7,15,17] The aim of our study was to describe the spectrum of phenotypes in patients with CCHS *PHOX2B* NPARM.

METHODS

The study is a retrospective case series of all patients with CCHS *PHOX2B* NPARM treated at Children's Hospital Los Angeles and Children's Healthcare of Atlanta between 2004 and 2018. Patients with CCHS *PHOX2B* NPARM were identified by reviewing the medical records of all patients with CCHS managed in the pulmonology and sleep medicine clinics at both institutions. De-identified demographic and clinical information was collected for all patients during the study period. The recorded data included *PHOX2B* gene variant; age and clinical features at presentation; duration and modality of assisted ventilation; presence of HD, NCT, and cardiac arrhythmia; and other clinical manifestations. All patients had confirmed *PHOX2B* gene mutation analysis. The study was approved by the Institutional Review Board at both institutions.

RESULTS

We identified 8 patients with 7 different heterozygous *PHOX2B* NPARM variants between 3 to 31 years of age. Five patients with CCHS *PHOX2B* NPARM were identified at Children's Hospital Los Angeles and 3 patients at Children's Healthcare of Atlanta. The *PHOX2B* genotype and clinical characteristics of our patients are summarized in **Table 1** and **Table 2**. All patients presented with respiratory depression in the first 2 months of life. Seven patients were diagnosed in infancy and 1 patient at 2 years of age. Patients 1 and 2 were previously reported with descriptions of their genotype and phenotype.[7] A brief description of the heterogeneous phenotypes is discussed here. In both institutions, annual cardiac ambulatory monitoring for at least 72 hours was performed to assess for cardiac dysrhythmia. In addition, periodic echocardiography was performed. Other surveillance monitoring was performed at the discretion of the patient's pulmonologist at both institutions.

Patient 3

Patient 3 was born at term and presented at birth with cyanosis briefly requiring supplemental oxygen. She had feeding difficulties, constipation, and at 2 weeks of age she developed abdominal distension and was diagnosed with HD. Rectal biopsy was performed, which showed long-segment HD. Following colostomy and subsequent pull-through surgeries, she developed cardiorespiratory arrest after receiving narcotic analgesics. In subsequent hospitalizations for gastrointestinal problems at 9 months of age, hypoxemia was noted, requiring initiation of supplemental oxygen. Polysomnography was performed that showed central sleep apnea, hypoxemia, and hypoventilation with end-tidal carbon dioxide ($ETCO_2$) above 50 mm Hg for 60% of the sleep time. CCHS was suspected and NPPV was initiated. She was discharged home on continuous NPPV with home nursing. At 15 months of age, she was weaned to NPPV only during sleep. At 20 months of age, *PHOX2B* genetic studies were performed and revealed a c.945A>G NPARM resulting in an abnormally

Table 2—Summary of clinical manifestations.

Patient	Assisted Ventilation	Age[a] at Initiation of Assisted Ventilation	Duration of Assisted Ventilation	Cardiac Arrhythmia	HD	Neurodevelopmental Delay	Neural Crest Tumor
1	PPV-T	1 day	Sleep	-	-	Speech delay	-
2	PPV-T	4 months	Sleep	Abnormal Holter study at 21 years requiring cardiac pacemaker	-	-	-
3	PPV-T	9 months	Sleep	-	Present	-	-
4	DP (day), PPV-T (sleep)	1 day	Full-time	-	Present	-	Adrenal ganglioneuroma (resected)
5	Oxygen	1.8 months	Sleep	-	-	-	-
6	PPV-T	1.4 months	Sleep	-	Present	-	-
7	PPV-T	2 months	Sleep	-	Present	Fine motor, speech delay	-
8	Oxygen	2 days	Sleep	-	-	-	-

[a]Age at initiation of any modality of assisted ventilation including intubation for mechanical ventilation with subsequent failure to wean off ventilatory support necessitating a tracheostomy, noninvasive positive pressure ventilation, or oxygen. DP = diaphragm pacing, HD = Hirschsprung's disease, PPV-T = positive pressure ventilation via tracheostomy, (-) = negative/normal.

elongated PHOX2B protein due to conversion of a stop codon to tryptophan. By 2.5 years of age, she was transitioned to tracheostomy and PPV-T during sleep. Despite dietary management and exercise, she developed progressive weight gain, leading to obesity at 6 years of age. Evaluation by a pediatric endocrinologist revealed acanthosis nigricans, normal thyroid and adrenal studies, elevated hemoglobin A1c (6.1%), and impaired glucose tolerance on oral glucose tolerance test. At 11 years of age, her weight was 94 kg (> 97th percentile) and body mass index was 38 kg/m^2 (> 97th percentile), which was attributed to exogenous obesity. Later that year, she was diagnosed with type 2 diabetes mellitus and metformin was initiated. Due to persistent constipation, colonic manometry was performed that revealed colonic myopathy in the residual colonic segment. Treatment with osmotic laxatives and stimulants led to improvement in constipation. Serial echocardiograms, Holter monitor studies, and imaging studies for neural crest tumors were normal.

Patient 4

Patient 4 was born at term gestation by cesarean delivery and developed apnea and cyanosis at birth. He was initially placed on NPPV, but due to persistent hypoxemia and hypercapnia, he required intubation and mechanical ventilation. Long-segment HD was diagnosed in the neonatal period, requiring ileostomy and gastrostomy tube for feeds. At 5 weeks of age, he was clinically diagnosed with CCHS, following which tracheostomy was performed for assisted ventilation. At 11 years of age, phrenic nerve electrodes were implanted for diaphragm pacing during the day while he continued to be ventilated via PPV-T at night. At 12 years of age, he required resection of the transverse colon due to persistent symptoms of HD. At 15 years of age, he was diagnosed with left adrenal ganglioneuroma that was incidentally detected during an emergency abdominal surgery for intestinal abscess and was surgically resected. Initial *PHOX2B* genetic studies showed normal 20/20 polyalanine repeats. Subsequently, *PHOX2B* gene sequencing revealed a missense c.428A>G NPARM that resulted in conversion of glutamic acid to arginine in the abnormal PHOX2B protein. He continues to require full-time continuous assisted ventilation delivered by diaphragm pacing while awake and PPV-T while asleep and gastrostomy tube feeds. Serial echocardiograms and Holter monitor studies were normal. NCT surveillance studies have not revealed any recurrence of ganglioneuroma. Parental *PHOX2B* studies were normal. At 27 years of age, he was employed, living independently, and received night-time home nursing.

Patient 5

Following an uncomplicated term delivery and neonatal course, patient 5 was hospitalized at 7 weeks of age with apnea and cyanosis. Evaluations revealed normal partial pressure of carbon dioxide on capillary blood gas and normal brain magnetic resonance imaging. He was diagnosed with an apparent life-threatening event and was discharged home on an apnea monitor. Due to recurrent alarms on home apnea monitor, he was readmitted 5 days later and polysomnogram was performed that showed severe obstructive sleep apnea (OSA) with apnea-hypopnea index (AHI) of 29.5 events/h, hypoxemia with baseline oxygen saturation (SpO_2) of 90–93%, nadir to 84%, and oxygen desaturation index of 39/h. There were no central apneas and peak $ETCO_2$ was 42 mm Hg, indicating absence of hypoventilation. Direct laryngoscopy and bronchoscopy were performed and showed mild laryngomalacia. Supplemental oxygen was initiated based on the sleep study. At 22 months of age, he was again hospitalized for apnea and oxygen desaturation during sleep.

Polysomnogram was repeated and showed severe OSA with AHI of 19.6 events/h and central sleep apnea (central AHI 5.1 events/h). The baseline SpO_2 on room air was 94–97%, nadir of 78%, and oxygen desaturation index was 64/h. $ETCO_2$ was 36–50 mm Hg, with peak of 58 mm Hg, and only 0.8% sleep time with $ETCO_2$ greater than 50 mm Hg. Supplemental oxygen was titrated to 2 L/min with improved SpO_2 of 95–98% and nadir of 92%. Due to recurrent apnea, *PHOX2B* gene sequence analysis was performed and revealed a heterozygous c.547G>T pathogenic variant in exon 3 of the *PHOX2B* gene. This nonsense variant resulted in substitution of guanine to thymine at nucleotide position 547 changing the amino acid from glutamic acid to a premature stop codon within exon 3. Echocardiogram and Holter monitoring were normal. Home oxygen therapy and pulse oximetry were continued and adenotonsillectomy was performed. At 3 years of age, polysomnogram was repeated and revealed resolved OSA (AHI 0.4 events/h) and central sleep apnea (central AHI 0.7 events/h). $ETCO_2$ was 48-50 mm Hg with a peak of 53 mm Hg, and only 1.4% sleep time with $ETCO_2$ greater than 50 mm Hg. $ETCO_2$ was suspected to be underestimated due to short sampling time. However, mild sleep-related hypoxemia persisted, requiring 1 L/min oxygen during sleep. He was evaluated by a gastroenterologist for constipation and studies did not reveal HD. He was diagnosed with functional constipation after a normal anorectal manometry study, and osmotic laxative therapy was initiated. Imaging studies for NCT, ophthalmologic evaluation, annual echocardiograms, and Holter monitor studies were normal. Due to concern for underrecognized hypoventilation, persistent sleep-related hypoxemia, and the diagnosis of CCHS, NPPV therapy was initiated. However, he was unable to tolerate NPPV during sleep and was referred to a sleep psychologist for desensitization strategies. Therefore, he continued to use oxygen during sleep with pulse oximetry.

Patient 6

Patient 6 was born at 38 weeks gestation by normal vaginal delivery and developed abdominal distension and bilious emesis on the second day of life. Abdominal radiograph showed gaseous distension of multiple loops of bowel suspicious for intestinal obstruction. Rectal biopsy established a diagnosis of HD and subtotal colectomy and colostomy were performed. She was extubated postoperatively and remained on room air until 6 weeks of age when she developed apnea and hypoxemia. Polysomnogram revealed obstructive and central sleep apnea, with obstructive and central AHI of 10 and 8 events/h, respectively. The average SpO_2 was 96%, nadir to 75%, and SpO_2 was less than 90% for 10% of the sleep time. $ETCO_2$ ranged from 40–62 mm Hg, and $ETCO_2$ was above 50 mm Hg for 40% of the study, diagnostic of sleep-related hypoventilation. Flexible nasolaryngoscopy, echocardiogram, and brain magnetic resonance imaging were normal. *PHOX2B* gene sequence analysis showed a heterozygous previously undescribed variant, c.432_433delGTinsTC caused by a 2-nucleotide deletion and a 2-nucleotide insertion within exon 3 of the *PHOX2B* gene. She was ventilated by PPV via endotracheal tube until 8 weeks of age, when she underwent tracheostomy for ventilation access. The following month, she underwent colostomy take-down and pull-through surgery for HD. She was discharged home on continuous PPV-T at 3 months of age. At 10 months of age, she was weaned to PPV-T only during sleep. Annual imaging studies for NCT, echocardiograms, and Holter monitor studies were normal.

Patient 7

Patient 7 was born at 38 weeks gestation by cesarean delivery. He developed apnea and feeding difficulties at birth requiring admission to the neonatal intensive care unit. He did not require oxygen or assisted ventilation in the neonatal period and was discharged home when apneas resolved at 1 week of age. Feeding difficulties persisted in the first 2 months of life with coughing and emesis after feeds. At 2 months of age, he presented to the emergency department with history of cough, nasal congestion, apnea, and duskiness. He was admitted in acute hypoxemic and hypercapnic respiratory failure due to rhino/enterovirus requiring high-flow nasal cannula therapy. He had SpO_2 of 84%, partial pressure of carbon dioxide of 63 mm Hg, and chest radiograph showed right upper lobar consolidation. He continued to have recurrent apneas despite antibiotics, radiographic resolution of consolidation, nasogastric tube feeds, and NPPV subsequently requiring intubation and mechanical ventilation. He failed 3 trials of extubation with ensuing apnea, hypoxemia, and hypercapnia. Echocardiogram and brain magnetic resonance imaging were normal, and endoscopic airway evaluation showed mild bilateral bronchomalacia and airway edema. At 3 months of age, *PHOX2B* genetic studies revealed a heterozygous c.448C>T variant on exon 3 of the *PHOX2B* gene. This missense variant resulted in a cytosine to thymine substitution at nucleotide position 448, changing the amino acid at codon 150 from arginine to cysteine. At 4 months of age, he underwent tracheostomy for ventilator access and gastrostomy tube placement. Despite absence of symptoms of HD, barium enema was performed and was indicative of HD. Subsequently, rectal biopsy was performed that revealed absence of ganglion cells confirming the diagnosis of rectosigmoid HD and endorectal pull-through surgery was performed. He was discharged home on continuous PPV-T and gastrostomy tube feeds at 6 months of age. He was weaned to PPV-T only during sleep at 11 months of age and at 1 year of age, gastrostomy tube was removed after a normal videofluoroscopic swallow study. At 2 years of age, neurodevelopmental evaluation revealed fine motor and speech delay. Echocardiograms, Holter monitoring, and studies for NCT were normal.

Patient 8

Patient 8 was born at 37 weeks gestational age by cesarean delivery and required hospitalization for feeding problems. She had mild hypotonia and intermittent desaturations requiring initiation of supplemental oxygen. Echocardiogram resulted normal. At 3 weeks of age, she was discharged home on continuous supplemental oxygen that was weaned to sleep use only at 3 months of age. She had a polysomnogram that showed mild OSA (AHI 3.1 events/h), baseline SpO_2 of 98%, SpO_2 less than 90% for 3% of sleep time, and nadir to 70% while on room air. She was unable to tolerate the $ETCO_2$ cannula during the study to assess for hypoventilation. Flexible nasolaryngoscopy showed normal findings. At 6 months of age, she was hospitalized for pneumonia

when she presented with cough, wheezing, rhinorrhea, hypoxemia, and persistent feeding difficulties. Aspiration was suspected, and videofluoroscopic swallow study showed multiple deep laryngeal penetrations with thin liquids alleviated by thickening liquids. Her serum bicarbonate level was normal (24 mEq/L). During the hospitalization, bradypnea and oxygen desaturations were noted during sleep while on baseline oxygen requirement of 0.25 L/min. Due to uncertain etiology of hypoxemia and bradypnea, *PHOX2B* genetic studies were performed and revealed a previously undescribed heterozygous c.242-1G>A splice variant. At 2 years, polysomnogram was repeated and showed severe OSA (AHI 12.4 events/h), baseline SpO_2 of 95%, nadir to 75%, and SpO_2 less than 90% for 1.2% of sleep time. $ETCO_2$ ranged from 30 to 55 mm Hg, and $ETCO_2$ was above 50 mm Hg for 32% of sleep time, diagnostic of sleep-related hypoventilation. She was placed on NPPV, but she was unable to tolerate it. She underwent adenotonsillectomy, and repeat polysomnogram showed residual moderate OSA (AHI 9 events/h), baseline SpO_2 of 95%, SpO_2 less than 90% for 0.7% of sleep time, and nadir to 80% while on room air. $ETCO_2$ ranged from 30 to 54 mm Hg, and $ETCO_2$ was above 50 mm Hg for 14% of sleep time. The persistent OSA was attributed to exogenous obesity (both weight and body mass index > 99th percentile), and NPPV was tried again due to concern for potential progression of hypoventilation. However, she was unable to tolerate NPPV despite several attempts. She continued to use oxygen with pulse oximetry during sleep. She did not have HD but had functional constipation. Echocardiogram, Holter monitoring, imaging studies for NCT, and parental *PHOX2B* studies were normal.

DISCUSSION

Our study shows that patients with CCHS *PHOX2B* NPARM can have a range of respiratory abnormalities from sleep-disordered breathing, central sleep apnea, to alveolar hypoventilation and need for ventilatory support as well as ANS involvement. We identified only 1 patient with a severe phenotype manifested by requiring continuous assisted ventilation, HD, and NCT. Five (63%) patients required PPV-T during sleep only. More significantly, we describe 2 patients requiring oxygen supplementation during sleep only without HD or NCT. Four (50%) patients had HD, and only 1 patient had a cardiac pacemaker due to bradyarrhythmia. Thus, our study adds to the growing literature describing the heterogeneous phenotypes in patients with CCHS *PHOX2B* NPARM variants and suggests that not all NPARM produce severe disruption of *PHOX2B* function as previously reported.[1,6]

In general, patients with NPARM require continuous assisted ventilation.[1] In our study, only 1 patient required continuous assisted ventilation and 5 patients required assisted ventilation only during sleep. Moreover, several case studies in patients with NPARMs have now reported a relatively milder respiratory phenotype requiring assisted ventilation only during sleep, with 2 studies reporting children with NPARMs requiring only oxygen during sleep.[7–9,11,13,16–18] Unger et al[9] reported an infant with HD, central apnea, and mild hypoventilation treated with only supplemental oxygen. Byers et al[8] reported 3 patients with the same NPARM and relatively mild and variable respiratory phenotype wherein each patient required either no respiratory support, oxygen, or NPPV during sleep. In their study, a patient initially treated with only supplemental oxygen later developed profound central sleep apnea and OSA with associated hypoxemia and hypercapnia requiring initiation of NPPV during sleep. In our study, we identified 2 patients (patients 5 and 8) without hypoventilation managed with only oxygen during sleep. It is possible these patients may progress to develop hypoventilation similar to the Byers et al[8] study, emphasizing the importance of periodic polysomnography. Sleep studies in our patients identified OSA, central sleep apnea, and hypoxemia without significant hypercapnia following treatment of OSA. Therefore, hypoventilation may not always be the hallmark of CCHS as previously reported.[8,9,19] With increased awareness of CCHS and availability of *PHOX2B* genetic studies, patients with atypical presentations and variable phenotypes are being identified, leading to the dilemma of formulating optimal ventilatory management strategies. At initial diagnosis during infancy, the current guideline recommends PPV-T to ensure optimal oxygenation, ventilation, and neurocognitive outcomes.[1] Based on the existing case studies and our own study, mechanical assisted ventilation via tracheostomy may not be necessary in all patients with less severe respiratory phenotypes.[8,9] In general, assisted ventilation is indicated when patients develop sleep-disordered breathing or hypoventilation. Assisted ventilation strategies may need to be individualized based on the severity of ventilatory disturbances. Nevertheless, even patients with relatively mild or variable phenotypes require close clinical follow-up, including periodic polysomnography, as the longitudinal evolution of ventilatory and ANS abnormalities with different NPARM is unclear.[8,9]

Although most patients with CCHS present in the neonatal period, patients may present later in infancy, childhood, and even during adulthood.[1,7,20] In some patients with late-onset CCHS, respiratory failure or unexplained hypoventilation may occur following a respiratory infection or exposure to anesthesia.[1] In our study, 6 patients developed respiratory depression in the neonatal period and 2 patients presented later in infancy. Interestingly, patient 7 developed apnea at birth that spontaneously resolved and later presented with persistent respiratory failure at 2 months of age following a pneumonia leading to the diagnosis of CCHS. Indeed, studies have identified individuals diagnosed with CCHS *PHOX2B* NPARM during childhood and even during adulthood.[11,21,22] This phenotypic variability in onset of ventilatory abnormalities may be due to environmental factors or modifier genes that alter the activity or expression of *PHOX2B*.[20]

HD is more prevalent in patients with NPARM (80%) compared to those with PARM (19%).[1] However, in our study only 4 (50%) patients had HD and 2 patients had functional constipation. CCHS management guidelines recommend evaluations for HD by barium enema or manometry and rectal biopsy in patients with constipation, and recommend against systematic investigations in asymptomatic patients.[1,19] However, patient 7 was diagnosed with HD despite absence of symptoms of HD. In this patient, barium enema was performed solely based on the clinical

association of HD with NPARM and was indicative of HD. This case highlights the importance of evaluating patients with NPARM for HD despite the absence of symptoms. In our study, 4 patients had feeding difficulties at birth and 2 patients required gastrostomy tube feeding. In addition, patient 8 had an abnormal swallow study requiring thickening of feeds. Therefore, infants with CCHS, particularly those with feeding difficulties, demand formal evaluation to determine safety of oral feeding and may require gastrostomy tube temporarily for adequate nutrition.[23,24]

NCTs are also more prevalent in patients with NPARM (41%) compared to those with PARM (1%).[1] In our study, only 1 patient developed an adrenal ganglioneuroma requiring resection at 15 years of age. Due to the reported high prevalence of NCT in patients with NPARM, delayed presentation, and potential adverse outcomes, serial evaluations for NCT should be performed.[1,19] In 2008, Gronli et al[25] reported an association between the size of polyalanine expansion in patients with PARM and the duration of R-R interval on Holter monitoring requiring implantation of cardiac pacemaker. However, the risk of cardiac arrhythmias in individuals with NPARM remained unascertained.[1] Indeed, a recent study showed that all patients with CCHS may be at risk for cardiac arrhythmia regardless of clinical symptoms or type of *PHOX2B* variant.[3] In our study, patient 2 had an abnormal Holter monitoring requiring cardiac pacemaker implantation at 22 years of age.[7] As sinus pauses can develop at any age including adulthood, even asymptomatic patients with CCHS require routine annual cardiac monitoring studies.[1,3] Our findings reiterate that all the clinical features of CCHS do not manifest at birth. Clinical features such as cardiac arrhythmia, hypoventilation, and NCT can manifest at any age in patients with CCHS emphasizing the importance of routine surveillance for these conditions.[1,7,8,19]

Among the NPARM, frameshift mutations (78%) are reported to be the most common.[1,6] Most reported NPARM are located at the end of exon 2 or exon 3 of the *PHOX2B* gene.[6] NPARM are associated with heterogeneous phenotypes, variable penetrance, and expressivity.[19] The phenotypic variability may be caused by unknown gene modifiers.[20,26] This possibility may explain the phenotypic variability seen in the family of patients 1 and 2 in our study. The missense variant c.245C>T in patient 1 was inherited from her asymptomatic mother. The affected maternal aunt (patient 2) inherited the same variant from her asymptomatic mother who had mosaicism. Different NPARM in the *PHOX2B* gene lead to different levels and mechanisms of cellular dysfunction, which have important implications for the severity of each patient's phenotype. Cain et al[13] reported that nonsense pathogenic variants in exon 1 of the *PHOX2B* gene escape nonsense-mediated decay (a cellular surveillance mechanism that prevents synthesis of abnormal proteins with potentially toxic effects) and produce truncated proteins that are functionally distinct compared to those produced by the PARM. Therefore, the type of NPARM and their respective location on the *PHOX2B* gene may play a critical role in the severity of phenotypes displayed by each patient. PARM and NPARM have different consequences on the PHOX2B protein and its function; however, the molecular effects of NPARM are not fully understood.[26–29] Due to the broad phenotypic spectrum reported in patients with NPARM, clinicians should thoroughly assess each patient's phenotype and perform routine surveillance studies for ventilatory abnormalities, cardiac arrhythmias, and NCT in accordance with established guidelines.[1,19]

In summary, we report that patients with CCHS *PHOX2B* NPARM can have relatively mild and heterogeneous phenotypes. Based on the variable NPARM phenotypes seen in our study and the existing literature reporting a broad spectrum of phenotypes, close monitoring of each patient with CCHS *PHOX2B* NPARM is essential to identify early manifestations of the associated comorbidities and implement a plan of care that is individualized for each patient.

ABBREVIATIONS

AHI, apnea-hypopnea index
ANS, autonomic nervous system
CCHS, congenital central hypoventilation syndrome
$ETCO_2$, end-tidal carbon dioxide
HD, Hirschsprung's disease
NCT, neural crest tumor
NPARM, nonpolyalanine repeat mutation
NPPV, noninvasive positive pressure ventilation
OSA, obstructive sleep apnea
PARM, polyalanine repeat expansion mutation
PHOX2B, paired-like homeobox 2B
PPV-T, positive pressure ventilation via tracheostomy
SpO_2, oxygen saturation

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All authors have seen and approved this manuscript. Work for this study was performed at Children's Hospital Los Angeles and Children's Healthcare of Atlanta. The authors report no conflicts of interest.