An Official ATS Clinical Policy Statement: Congenital Central Hypoventilation Syndrome

Genetic Basis, Diagnosis, and Management

Debra E. Weese-Mayer, Elizabeth M. Berry-Kravis, Isabella Ceccherini, Thomas G. Keens, Darius A. Loghmanee, and Ha Trang, on behalf of the ATS Congenital Central Hypoventilation Syndrome Subcommittee

THIS OFFICIAL CLINICAL POLICY STATEMENT OF THE AMERICAN THORACIC SOCIETY (ATS) WAS APPROVED BY THE ATS BOARD OF DIRECTORS SEPTEMBER 2009.

Background: **Congenital central hypoventilation syndrome (CCHS) is characterized by alveolar hypoventilation and autonomic dysregulation.**

Purpose: **(*1*) To demonstrate the importance of *PHOX2B* testing in diagnosing and treating patients with CCHS, (*2*) to summarize recent advances in understanding how mutations in the *PHOX2B* gene lead to the CCHS phenotype, and (*3*) to provide an update on recommendations for diagnosis and treatment of patients with CCHS.**

Methods: **Committee members were invited on the basis of their expertise in CCHS and asked to review the current state of the science by independently completing literature searches. Consensus on recommendations was reached by agreement among members of the Committee.**

Results: **A review of pertinent literature allowed for the development of a document that summarizes recent advances in understanding CCHS and expert interpretation of the evidence for management of affected patients.**

Conclusions: **A *PHOX2B* mutation is required to confirm the diagnosis of CCHS. Knowledge of the specific *PHOX2B* mutation aids in anticipating the CCHS phenotype severity. Parents of patients with CCHS should be tested for *PHOX2B* mutations. Maintaining a high index of suspicion in cases of unexplained alveolar hypoventilation will likely identify a higher incidence of milder cases of CCHS. Recommended management options aimed toward maximizing safety and optimizing neurocognitive outcome include: (*1*) biannual then annual in-hospital comprehensive evaluation with (*i*) physiologic studies during awake and asleep states to assess ventilatory needs during varying levels of activity and concentration, in all stages of sleep, with spontaneous breathing, and with artificial ventilation, and to assess ventilatory responsiveness to physiologic challenges while awake and asleep, (*ii*) 72-hour Holter monitoring, (*iii*) echocardiogram, (*iv*) evaluation of ANS dysregulation across all organ systems affected by the ANS, and (*v*) formal neurocognitive assessment; (*2*) barium enema or manometry and/or full thickness rectal biopsy for patients with a history of constipation; and (*3*) imaging for neural crest tumors in individuals at greatest risk based on *PHOX2B* mutation.**

Keywords: respiratory control; autonomic dysregulation

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OVERVIEW

In 1999 the American Thoracic Society published the first Statement on Congenital Central Hypoventilation Syndrome (CCHS) (1). Since then, the world of CCHS has exploded with (*1*) the discovery that the paired-like homeobox 2B (*PHOX2B*) gene is the disease-defining gene for CCHS (2–5); (*2*) identification of an autosomal dominant inheritance pattern (3, 5–7); (*3*) demonstration of a *PHOX2B* genotype–CCHS phenotype relationship pertinent to ventilatory dependence (3, 5), facial dysmorphology (8), cardiac asystoles (9), and Hirschsprung disease and neuroblastoma (6, 7); (*4*) identification of *PHOX2B* mutations in CCHS adults and older children (10–17) whose diagnosis was "missed" or not apparent in the neonatal period, infancy, and early childhood; (*5*) documentation of mosaicism in 5 to 10% of parents of children with CCHS (3, 6); and (*6*) improved understanding of the specific mechanisms whereby *PHOX2B* results in the CCHS phenotype (6, 18–21). The purpose of a new ATS statement on CCHS is to aid the clinician in optimizing patient care that will be specifically tailored to knowledge of the individual *PHOX2B* genotype/mutation, and to offer genetic counseling. Finally, all management options will be addressed with the long-term goals of improving quality of life for *PHOX2B* mutation-confirmed individuals with CCHS and to gain a better understanding of the autonomic nervous system (ANS) in health and disease. These include (*1*) biannual then annual in-hospital evaluation with (*i*) physiologic studies during awake and asleep states to assess ventilatory needs during varying levels of activity and concentration, in all stages of sleep, with spontaneous breathing, and with artificial ventilation,

and to assess ventilatory responsiveness to physiologic challenges while awake and asleep, (*ii*) 72-hour Holter monitoring, (*iii*) echocardiogram, (*iv*) assessment of ANS dysregulation across all organ systems affected by the ANS, and (*v*) formal neurocognitive assessment; (*2*) barium enema or manometry and/or full thickness rectal biopsy for patients with a history of constipation; and (*3*) imaging for neural crest tumors in individuals at greatest risk based on *PHOX2B* mutation.

METHODS

Committee members were invited on the basis of their expertise in the care of patients with CCHS in terms of clinical care, clinical research, or basic science investigation. Representation was included from the pediatric and adult communities. The intent was for international representation. Committee members were asked to review the current state of the science by independently completing literature searches using Pub Med and OVID. Each committee member was asked to assess the identified literature, provide a critique of articles, and rate the importance of individual articles. The most highly ranked articles and the critiques were integrated into the working document. Consensus on the recommendations was reached among the members of the Committee.

OBJECTIVES

1. To inform the practitioner, parent, caregiver, and health care provider that a *PHOX2B* mutation is requisite to confirmation of a diagnosis of CCHS.
2. To improve general knowledge regarding *PHOX2B* as the disease-defining gene for CCHS. The reader will learn that (*i*) approximately 90% of individuals with the CCHS phenotype are heterozygous for a polyalanine expansion repeat mutation in the *PHOX2B* gene, (*ii*) approximately 10% of individuals with CCHS are heterozygous for a missense, nonsense, or frameshift mutation in the *PHOX2B* gene, (*iii*) other non-CCHS diagnoses should be sought if a *PHOX2B* mutation is not found.
3. To introduce the opportunity to anticipate the CCHS phenotype based on the *PHOX2B* genotype/mutation.
4. To educate clinicians that CCHS is no longer diagnosed exclusively in the newborn period, as it is now described among toddlers, children, and adults.
5. To focus on the autosomal dominant inheritance pattern of the *PHOX2B* mutation in CCHS, the finding of mosaicism in 5 to 10% of parents, and the importance of testing both parents of each subject with CCHS.
6. To improve understanding of the specific mechanisms whereby *PHOX2B* results in the CCHS phenotype.
7. To update information regarding available treatment and home health care options.
8. To recognize that CCHS is a model for translational and transitional autonomic medicine. In addition to using the *PHOX2B* genetic mutation to optimize patient management, there will be a need for clinicians to continue to care for these special patients as they mature into adulthood.

THE STATEMENT

Historical Background and General Information on CCHS

CCHS was first described in 1970 by Robert Mellins and colleagues (22). Despite a multitude of case reports, large series were not published until 1992 (23). The 1999 ATS Statement on CCHS estimated "roughly 160 to 180 living children with CCHS worldwide" but advised that these numbers "are considered to be an underestimate" (1). In 2009, the collective laboratories from the United States, France, Italy, Japan, Germany, Taiwan, China, The Netherlands, Chile, the UK, and Australia have now diagnosed nearly 1,000 cases with *PHOX2B* mutation-confirmed CCHS. Even now, this is recognized to be an underestimate, as individuals with the milder phenotype are underdiagnosed. Although CCHS is characteristically diagnosed during the newborn period, recent reports indicate that individuals can be diagnosed in childhood (5, 6, 17, 24–26) and adulthood (10–17, 26), depending upon the *PHOX2B* genotype and the intellectual inquisitiveness of the patient, family, and medical team. Regardless of age at presentation, individuals with CCHS will be clinically diagnosed in the absence of primary lung, cardiac, or neuromuscular disease or an identifiable brainstem lesion that might account for the *entire* phenotype inclusive of the autonomic nervous system dysregulation (ANSD). Individuals with CCHS characteristically have diminutive tidal volumes and monotonous respiratory rates awake and asleep (1), although the more profound alveolar hypoventilation occurs primarily sleep. As a result of the hypoventilation, these individuals will become hypoxemic and hypercarbic but typically lack the responsiveness to these endogenous challenges in terms of ventilation and arousal during sleep, and they lack the perception of asphyxia during wakefulness with and without exertion (1). Conditions associated with CCHS reflecting anatomic ANSD include Hirschsprung disease (HSCR) and tumors of neural crest origin in addition to a spectrum of symptoms compatible with physiologic ANSD, including diminished pupillary light response, esophageal dysmotility, breath-holding spells, reduced basal body temperature, sporadic profuse sweating, lack of perception to dyspnea, altered perception of anxiety, and lack of physiologic responsiveness to the challenges of exercise and environmental stressors (1, 23, 27–39). CCHS is a lifelong disease, which raises key questions including: (*1*) will the phenotype change with advancing age based on the *PHOX2B* mutation, age at diagnosis, and adequacy of management? and (*2*) will intervention strategies be effective considering the nature of the mutation and its timing in terms of embryologic development? As the aim of this Statement is not to provide an exhaustive review of CCHS, the reader is referred to recent reviews (www.genereviews.org and References 40 and 41).

PHOX2B: The Disease-defining Gene for CCHS

Hints toward the familiality of CCHS emerged between the 1980s and 2001. Familial recurrence data include one report each of affected monozygotic female twins (42), sisters (43), male-female siblings (23, 44), and male-female half siblings (45) with CCHS. In the pre-*PHOX2B*/CCHS era, five women diagnosed with CCHS in their own childhoods gave birth to two infants with definite CCHS, one with likely CCHS confounded by severe immaturity and bronchopulmonary dysplasia, and one with later-onset CCHS (46, 47). A report of a child with CCHS born to a woman who had neuroblastoma as an infant (48) added to the premise of a transmitted genetic component in the phenotypic spectrum of ANSD and CCHS. Furthermore, ANSD was studied in a case-control family design (27, 28), which provided important confirmatory evidence for a genetic basis to CCHS and is therefore regarded as the most severe manifestation of a general ANSD (1, 27, 44), although the role of *PHOX2B* in the broader category of ANSD remains unknown.

Most early studies, undertaken in pursuit of the genetic basis for CCHS, were restricted to genes known to be related to HSCR. Twenty patients were reported to have protein-altering

mutations in receptor tyrosine kinase (*RET*) (49–53), glial cell–derived neurotrophic factor (*GDNF*) (49), endothelin signaling pathway 3 (*EDN3*) (52, 54), brain-derived neurotrophic factor (*BDNF*) (55), human aschaete-scute homolog gene (*HASH1*) (4, 56), paired-like homeobox gene 2A (*PHOX2A*) (4), *GFRA1* (4), bone morphogenic protein 2 (*BMP2*) (3), and endothelin converting enzyme 1 (*ECE1*) (3). Three other reports indicate an absence of *RET* (57) and *RNX* mutations (58, 59).

In 2003, *PHOX2B* was found to be the disease-defining gene for CCHS (2, 3). *PHOX2B* encodes a highly conserved homeodomain transcription factor known to play a key role in the development of ANS reflex circuits in mice (60, 61). *PHOX2B* contains a repeat sequence of 20 alanines in exon 3, which Amiel and colleagues reported to contain in-frame duplications of 15 to 27 nucleotides, leading to expansion of the repeat tract to 25 to 29 alanines on the affected allele in 18 of 29 (62%) French CCHS cases (2). These expansions appeared to be *de novo* insofar as they were not present in eight sets of parents of the CCHS cases. Two of 29 (7%) CCHS cases had frameshift mutations. Collectively, 69% of the 29 French patients were heterozygous for a *PHOX2B* mutation, but none of the controls had *PHOX2B* mutations. Amiel and colleagues (2) also demonstrated *PHOX2B* expression in early human embryos in both central autonomic neuron circuits and in peripheral neural crest derivatives.

Concurrent to the French studies, Weese-Mayer and colleagues (3) focused on genes involved in the early embryology of the ANS (mammalian aschaete-scute homolog-1 [*MASH1*], *BMP2*, engrailed-1 [*EN1*], *TLX3*, *ECE1*, endothelin-1 [*EDN1*], and *PHOX2A*). Although no novel disease-causing mutations were found in any of these genes in a cohort of 67 CCHS cases, Weese-Mayer and colleagues (3) identified heterozygous *PHOX2B* exon 3 polyalanine repeat expansions of 25 to 33 repeats in 65 of 67 (97%) children with the CCHS phenotype. Of the two remaining CCHS cases, a nonsense mutation (premature stop codon) in *PHOX2B* was identified in one patient and the other was later found to have a polyalanine repeat expansion in *PHOX2B* after a sample mix-up at the lab of origin was resolved (7). Collectively, Weese-Mayer and colleagues (3) identified mutations in exon 3 of the *PHOX2B* gene in 100% of the 67 children with the CCHS phenotype, indicating that *PHOX2B* is the disease-defining gene in CCHS. None of the *PHOX2B* expansion mutations were present in 67 gender/ethnicity-matched controls. This study also noted (*1*) an association between polyalanine expansion length and severity of autonomic dysfunction; (*2*) mosaicism in 4 of 97 parents of CCHS cases, suggesting that not all *PHOX2B* mutations occur *de novo*; (*3*) autosomal dominant inheritance of the *PHOX2B* mutation and the CCHS phenotype from CCHS cases; and (*4*) autosomal dominant inheritance of the *PHOX2B* mutation from mosaic parents. Furthermore, these authors (3) established the first clinically available assay for the diagnosis of CCHS using a simple and accurate method for detecting and sizing the repeat sequence associated with the polyalanine tract expansion (patented; Rush University Medical Center, Chicago, IL; patent donated to charitable trust and proceeds from *PHOX2B* Screening Test support CCHS research), which could also be used for prenatal diagnosis, family testing, and diagnosis of individuals with relevant symptoms.

Subsequent to the above studies, *PHOX2B* polyalanine repeat expansions were found in 4 (40%) and a *PHOX2B* insertion frameshift mutation in 1 (10%) of 10 CCHS cases in Japan (4). The expansion was shown to be *de novo* in 2 cases. Sasaki and colleagues (4) used the same methodology as the French (2) and also underdetected *PHOX2B* expansion cases as reported in 2005 (62). In 2004, Matera and colleagues (5) identified heterozygous *PHOX2B* polyalanine expansion mutations of 25 to 33 repeats in 21 (88%) and heterozygous frameshift mutations in 2 (8%) of 24 CCHS cases from Italy, Germany, and The Netherlands. This study confirmed the correlation between the size of the *PHOX2B* expanded allele and the severity of the respiratory phenotype and associated symptoms (3). Matera and colleagues also demonstrated that in standard polymerase chain reactions the CCHS-associated expanded allele, especially those with 30 to 33 alanines, can remain undetected due to the GC-rich polyalanine region of *PHOX2B*; thus, the *PHOX2B* mutation rate may be underestimated as a result of the amplification-induced allele dropout. Using assays designed to amplify GC-rich regions, Trang and colleagues (63) (re)analyzed 34 of the French patients and identified a *PHOX2B* mutation in 91% of the cases, and Trochet and colleagues (6) found *PHOX2B* mutations in 93% of 174 subjects with CCHS from multiple nationalities, including 7 of 9 "mutation-negative" patients reported by Amiel and colleagues in 2003 (2). Berry-Kravis and colleagues (7) and Weese-Mayer and colleagues (64) reported *PHOX2B* mutations in 184 subjects (in 2006) and collectively more than 350 subjects (in 2008) with CCHS, respectively (100% sensitivity and specificity of detection), in a cohort primarily from the United States, with 10% of patients from abroad.

As previously summarized (40, 41) (www.genereviews.org), the range for the number of repeats in the *PHOX2B* polyalanine expansion on the affected allele in patients with CCHS is 24 to 33 (2–7, 13, 15–17, 25, 26, 65–70). Polyalanine repeat expansion mutations (PARMs) were not found in 482 controls from the above-cited publications nor among 1520 healthy individuals in Taiwan (67). In-frame contraction variants (with 7, 13, 14, or 15 repeats in the polyalanine repeat tract) have been reported in three CCHS cases (3, 7, 71) who harbor an additional polyalanine expansion repeat mutation or nonpolyalanine repeat mutation (NPARM) but are also found in approximately 3% of seemingly normal controls (2, 3, 5, 72, 73), CCHS parents (3, 16, 71), and a small subset of patients with vague symptoms suggestive of autonomic dysregulation and/or sporadic hypoventilation, or apparent life-threatening events but not the constellation of symptoms characteristic of CCHS (71). A *PHOX2B* polyalanine repeat expansion mutation segregating with disease was observed in 9 of 16 patients with *RET*, *GDNF*, *BDNF*, *HASH1*, and G*FRA1* coincidental mutations, thus indicating that *PHOX2B* is the disease-defining gene in these children.

PHOX2B Mutations in CCHS

A mutation in the *PHOX2B* gene is requisite to a diagnosis of CCHS. Over 90% of CCHS cases will be heterozygous for an in-frame PARM coding for 24 to 33 alanines in the mutated protein and producing genotypes of 20/24 to 20/33 (the normal genotype would be referred to as 20/20). The remaining approximately 10% of patients with a classical CCHS phenotype will be heterozygous for an NPARM (74) (including missense, nonsense, and frameshift) in the *PHOX2B* gene. The 20/25, 20/26, and 20/27 genotypes are the most common, although growing numbers of even the less-common mutations are being identified monthly (*see* a histogram of all published data as well as all current data from the authors as of late 2009 in Figure 1).

NPARMs (74) have been reported in association with CCHS by groups in the United States (3, 7, 20, 41, 64), Italy (5, 16, 18), Japan (4), France (2, 6, 13, 75), Germany (50, 69, 76), Australia (77), The Netherlands (78), China (79), and Taiwan (67, 80). Thus far, 76 individuals with CCHS and NPARMs in *PHOX2B* have been described worldwide, and mutations include pre-

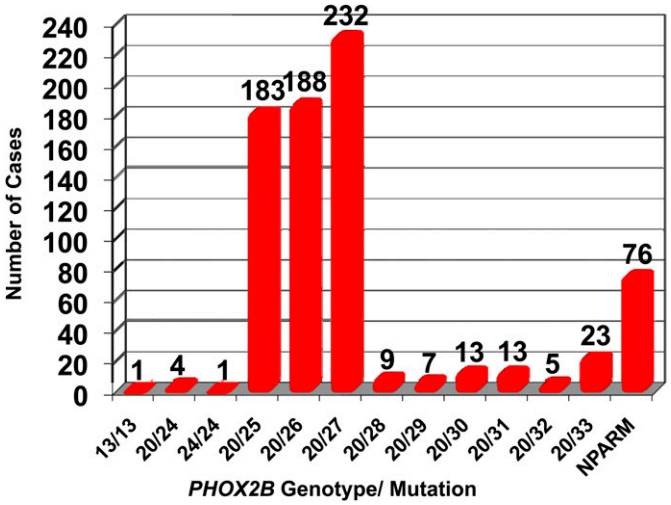

Figure 1. Number of *PHOX2B* polyalanine repeat mutations by genotype. These data represent all published literature as well as the provided current data from the Statement authors for the polyalanine repeat mutations (PARMs) and the nonpolyalanine repeat mutations (NPARMs). The most common genotypes are 20/25, 20/26, and 20/27. Adapted by permission from Reference 41.

dominantly frameshift mutations (59/76, 78%), but also nonsense (3/76, 4%), missense (12/76, 16%), and missense with stop codon alteration (2/76, 3%) (*see* Figure 2 for a schematic of all published data as well as all current data from the Statement authors). The majority of CCHS-associated NPARMs are found at the end of exon 2 or in exon 3 (Figure 2).

The majority of NPARMs occur *de novo* and produce very severe phenotypes with HSCR and extensive gut involvement, need for continuous ventilatory support, and increased tumor risk in those over 1 year of age (6, 7). Thus, the presence of extensive HSCR and a CCHS phenotype is a strong predictor of a *PHOX2B* NPARM. Recurrent 38 and 35 base pair deletions, causing frameshift from the polyalanine repeat throughout the protein, produce severe disease and have been identified by several groups in different countries. A minority of NPARMs are associated with a high incidence of HSCR but a milder physiologic CCHS phenotype, and incomplete penetrance in at least 3 families (7). A few similarly located frameshift mutations (618delC, 577delG) have been inherited and are variably penetrant in families (5, 7), suggesting that −1 frameshifts in this area may produce a milder cellular deficit than other frameshift mutations. The c.422G > A and c.428A > G mutations, leading to p.R141Q and p.Q143R, respectively, have also been found in several unrelated cases of CCHS and, together with the c.299G > T (p.R100L) mutation (20), are the only missense mutations yet identified in CCHS. The c.419C > A (p.A140E) has recently been reported in later-onset CCHS, both isolated and associated with HSCR (13, 81).

PHOX2B Genotype/CCHS Phenotype

Despite identification that *PHOX2B* is the disease-defining gene for CCHS in 2003, journals continue to publish research without (*1*) confirmation that all subjects with "CCHS" have *PHOX2B* mutations, (*2*) a distinction in data analysis between subjects with *PHOX2B* mutation-confirmed CCHS and children with other causes of hypoventilation, and (3) analysis of data in a *PHOX2B* genotype/CCHS phenotype format. Owing to the crucial role of *PHOX2B* in the development of the ANS, it is pertinent to hypothesize a relationship between *PHOX2B* genotype and the following aspects of the CCHS phenotype.

Continuous ventilatory dependence. There is a relationship between the genotype for PARMs and the need for continuous ventilatory dependence (3, 5, 7, 65). Specifically, individuals with the 20/25 genotype rarely require 24-hour per day ventilatory support; individuals with the 20/26 genotype have variable awake needs depending upon the level of activity; and individuals with genotypes from 20/27 to 20/33 typically require continuous ventilatory support. Later-onset cases with the 20/24 or 20/25 genotype (10, 11, 17) have the mildest hypoventilation, presenting primarily after exposure to respiratory depressants or severe respiratory infection, and are managed with nocturnal ventilatory support only. In contrast to the PARMs, most individuals with NPARMs require continuous ventilatory support (7) (Figure 3).

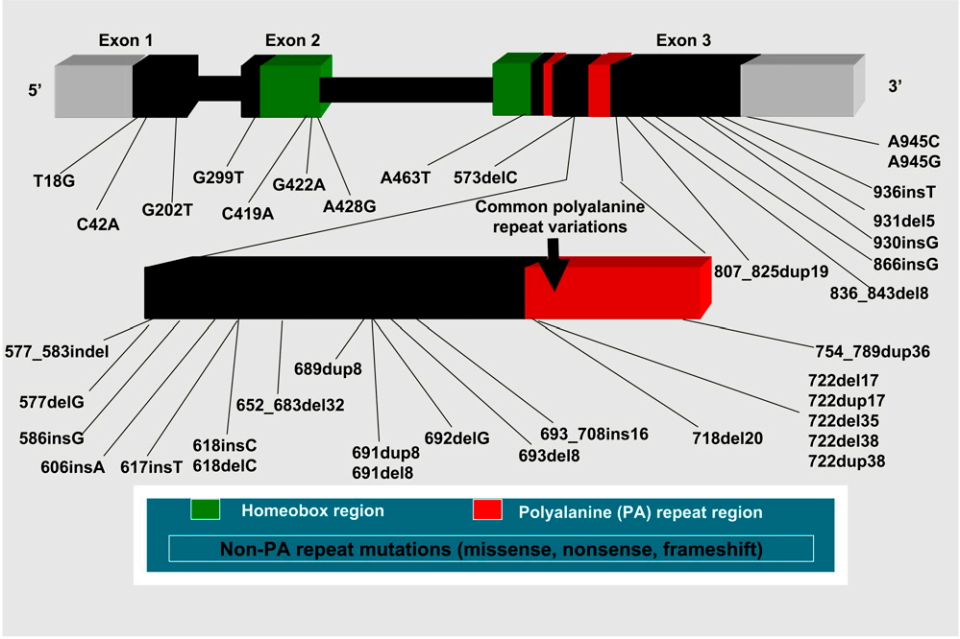

Figure 2. Schematic for the *PHOX2B* gene with location of all CCHS-associated mutations described to date in *PHOX2B.* All polyalanine repeat mutations (PARMs) are located within the second polyalanine stretch of exon 3. Nearly all thus far identified NPARMs are found at the 3′ end of exon 2 or in exon 3. These data represent all published literature and current data provided by the Statement authors (41). Reproduced by permission from Reference 41.

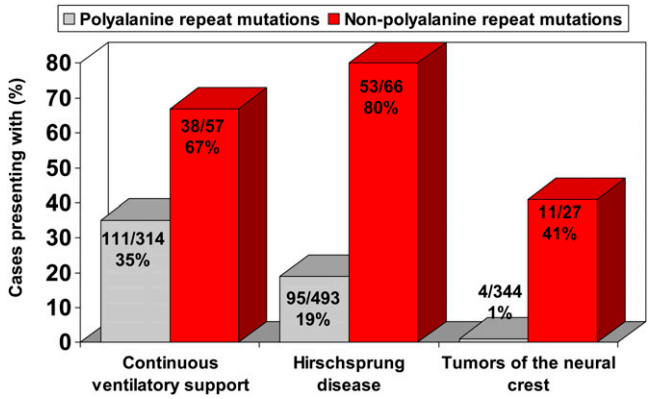

Figure 3. Rate of continuous ventilatory dependence, Hirschsprung disease, and tumors of the neural crest in congenital central hypoventilation syndrome (CCHS) cases with polyalanine repeat expansion mutations (PARMs) in *PHOX2B* compared with CCHS cases with non-polyalanine repeat expansion mutations (NPARMs) in *PHOX2B*. CCHS cases included in this figure were compiled from all known cases reported in the literature, including reports from groups in the United States, Italy, France, Japan, Germany, Taiwan, China, Australia, and The Netherlands as well as current information reported by the Statement authors, where adequate clinical information was available. Neural crest tumor data was derived from cases in which information was available and the child had survived at least the first year of life. All PARM cases with tumors had large (29–33 repeat) expansion mutations. Adapted by permission from Reference 41.

Hirschsprung disease. Long recognized to occur among 20% of cases of CCHS, HSCR is more clearly prevalent among cases of the NPARMs than the PARMs. Specifically, HSCR is reported in 87 to 100% of NPARMs in contrast to 13 to 20% of PARMs (6, 7, 65) (*see* Figure 3 for a histogram of all adequately detailed published data as well as all provided current data from the ATS Statement authors). Among the PARMs, there are no reports of HSCR occurring in subjects with the 20/25 genotype, and only rarely with the 20/26 genotype. A high occurrence of HSCR in individuals with the 20/27 genotype was reported in one cohort (6) but was not yet definitively confirmed in others. Recent studies further suggest that the *RET* gene may have a pivotal role as a modifier gene for the HSCR phenotype in patients with CCHS (53, 82).

Tumors of neural crest origin. Tumors of neural crest origin occur more frequently among individuals with NPARMs (50%) than among those with PARMs (1%) (6, 7, 65) (Figure 3) (all neuroblastomas). However, among PARMs, only subjects with the 20/29 and 20/33 genotypes (2, 3, 6, 7) have been identified to have tumors of neural crest origin (ganglioneuromas and ganglioneuroblastomas) thus far.

Cardiac asystoles. A recent report by Gronli and colleagues (9) identified a correlation between the most common PARMs (genotypes 20/25–20/27) and length of R-R intervals on Holter monitoring. Specifically, none of the children with the 20/25 genotype had sinus pauses of 3 seconds or longer. However, 19% of individuals with the 20/26 genotype and 83% of individuals with the 20/27 genotype had pauses of 3 seconds or longer. Similarly, cardiac pacemakers were implanted among 0% of the subjects with the 20/25 genotype, 25% of subjects with the 20/26 genotype, and 67% of subjects with the 20/27 genotype. Among the cases with the 20/26 and 20/27 genotypes who did not receive a cardiac pacemaker, two died suddenly and one had severe neurocognitive compromise. Further, one adult with the 20/25 genotype, diagnosed with CCHS in adulthood, had documented pauses of 8 seconds and longer (11) and the ATS Statement authors have been alerted to another later-onset adult with the 20/25 genotype and prolonged asystoles on Holter recording. These findings raise concern that individuals with the 20/25 genotype may be unaffected during childhood but, if not adequately managed or promptly diagnosed, may experience prolonged asystoles in adulthood. Risk to individuals with NPARMs remains unascertained.

Symptoms of ANSD. Weese-Mayer and colleagues and Patwari and colleagues (3, 83) demonstrated that an increased number of polyalanine repeats was associated with an increased number of symptoms of ANS dysregulation (Figure 4). Though these measures of ANSD were ascertained from review of medical records, scripted questionnaires, and physiologic assessment, they did not include specific tests to assess autonomic function. However, physicians and parents should expect more symptoms of ANSD among subjects with genotypes 20/27 to 20/33.

Facial dysmorphology. A report by Todd and colleagues (8) described a characteristic facies among children between the ages of 2 years and early adulthood that have CCHS and primarily among individuals with PARMs. The faces of subjects with CCHS were generally shorter and flatter, and typically showed an inferior inflection of the lateral 1/3 of the upper vermilion border (lip trait). Though not dysmorphic, the face is short relative to its width, resulting in the characteristic box-shaped face observed in CCHS. Eighty-six percent of the CCHS cases and 82% of controls were correctly predicted using five variables to characterize facies (upper lip height, biocular width, upper facial height, nasal tip protrusion, and the lip trait). A limited number of cases with higher numbers of repeats (only five cases with 30–33 repeats) may have precluded the identification of a significant correlation between the number of polyalanine repeats and measures of the CCHS facial phenotype.

Dermatoglyphics. Todd and colleagues (84) assessed dermatoglyphic pattern type frequency, left/right symmetry and genotype/phenotype correlation in CCHS. Dermatoglyphic pattern type frequencies were altered in cases of CCHS versus controls:

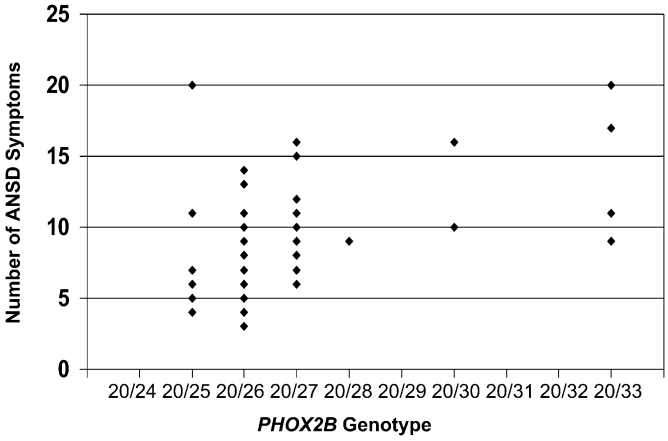

Figure 4. Number of autonomic nervous system dysregulation (ANSD) symptoms in congenital central hypoventilation syndrome (CCHS) cases versus *PHOX2B* genotype among 65 children with a polyalanine repeat expansion mutation (PARM). The number of symptoms of ANSD increases with the number of alanines in the PARM. Many subjects had identical numbers for ANSD symptoms and genotype, therefore the figure gives the illusion of fewer data points than expected for the cohort size. Measured genotype analysis (consisting of comparing the genotypic means by ANOVA) revealed a significant association between *PHOX2B* polyalanine repeat mutation length and number of symptoms of ANS dysregulation ($F = 2.93$, $df = 5$, $P = 0.021$). Adapted by permission from Reference 3.

increase of arches in females and ulnar loops in males, with the largest differences for the left hand and for individuals with both CCHS and HSCR, was reported. Dissimilarity scores between the CCHS and CCHS/HSCR cases, and between all female and all male cases were not significantly different. No significant association was found between the number of polyalanine repeats in the *PHOX2B* genotypic category and dermatoglyphic pattern frequencies in the CCHS study groups.

CCHS: Not Just for Babies

The term "congenital" as used historically in CCHS, connoted presentation in the newborn period. However, patients presenting outside of the newborn period with later-onset (LO)–CCHS have been described previously (5, 6, 10–17, 24–26, 85). In the context of (*1*) increased awareness of CCHS; (*2*) the discovery that *PHOX2B* is the disease-defining gene for CCHS; and *3*) the availability of clinical diagnostic testing for *PHOX2B* mutations, an increase in diagnosis of LO–CCHS with presentation in later infancy, childhood, and adulthood is anticipated.

LO-CCHS reflects the variable penetrance of the *PHOX2B* mutations with the genotypes 20/24 and 20/25 or rarely an NPARM that may require an environmental cofactor to elicit the phenotype. Careful review of the medical history for individuals "presenting" with alveolar hypoventilation after the newborn period often demonstrates signs and symptoms compatible with prior hypoventilation and other disorders of autonomic regulation from the newborn period. The diagnosis of LO–CCHS should be considered in cases of centrally mediated alveolar hypoventilation and/or cyanosis or seizures noted after (*1*) administration of anesthetics or CNS depressants, (*2*) recent severe pulmonic infection, or (*3*) treatment of obstructive sleep apnea. With a heightened clinical suspicion of LO–CCHS, the physician can expedite the diagnosis by promptly testing for a *PHOX2B* mutation, thereby averting potentially life-threatening decompensation as well as risk for neurocognitive compromise. Evaluation of later presentation cases requires a careful history with attention to past exposure to anesthesia or sedation, delayed "recovery" from a severe respiratory illness, and unexplained seizures or neurocognitive impairment. Also, review of digital frontal and lateral photographs (to evaluate for facies consistent with CCHS; adult males often have a moustache to conceal the "lip trait"), any electrocardiographic documentation of prolonged sinus pauses (ideally via 72-h Holter monitoring), any physiologic evaluations documenting ventilation while awake and while asleep (for hypercarbia and/or hypoxemia), a hematocrit and reticulocyte count (for polycythemia and response to hypoxemia), a bicarbonate level (for signs of compensated respiratory acidosis), or chest x-ray, echocardiogram, or electrocardiogram (for signs of right chamber enlargement or pulmonary hypertension) should be completed. In cases of constipation, a barium enema or manometry may be considered to exclude short segment HSCR. Patients diagnosed after the neonatal period would be termed LO–CCHS and can be distinguished from other syndromes of mild alveolar hypoventilation by the presence of a *PHOX2B* mutation.

The 20/24 genotype is likely underdiagnosed because of the subtle hypoventilation and potential need for environmental cofactors (17) or the homozygous condition to manifest the CCHS phenotype (66). Molecular analyses of *PHOX2B* in cohorts of individuals presenting with profound hypoventilation after anesthesia, sedation, or respiratory illness may identify additional patients with the 20/24 and 20/25 genotype. In so doing, other yet-unidentified environmental or genetic factors that might impact the variable penetrance of the 20/24 and 20/25 mutations, as well as the most recently described novel missense mutation (81), may be determined.

It is essential that practitioners distinguish LO–CCHS from rapid-onset obesity with hypothalamic dysfunction, hypoventilation, and autonomic dysregulation (ROHHAD) (86), a rare disorder first described in 1965 (87). Originally termed late-onset central hypoventilation syndrome with hypothalamic dysfunction (88), it was renamed in 2007 (86) to alert the practitioner to the typical sequence of presenting symptoms. In that same publication, Ize-Ludlow and colleagues clarified that ROHHAD is a distinctly different syndrome from CCHS as demonstrated by careful history and *PHOX2B* testing. Although fewer than 55 children have been described in the literature with this disorder (86, 88, 89), it is essential to recognize the phenotype as distinct from CCHS. Children with ROHHAD typically present between the ages of 1.5 and 7 years with rapid onset obesity (20–40 pound gain over 4–6 mo), followed by the recognition of other hypothalamic disorders including water imbalance, elevated prolactin levels, altered onset of puberty, and more. Nearly half of the children will experience a cardiorespiratory arrest after an intercurrent viral infection, then obstructive sleep apnea and hypoventilation will be noted. At variable times thereafter, symptoms of autonomic dysregulation including low body temperature, cold hands and feet, severe bradycardia, decreased pain perception, among others, will become apparent. At any point in the disease manifestation as many as 40% of children will demonstrate a tumor of neural crest origin, often associated with scoliosis. Behavioral disorders, strabismus, and abnormal pupillary responses are all reported in ROHHAD. These children can often be supported with mask ventilation only at night, but a subset of them will require 24-hour per day ventilation via tracheostomy. Although ongoing studies into the possible causes of ROHHAD are underway, the specific cause for this disorder has not been identified. As there is no genetic testing available for this disorder, the diagnosis of ROHHAD is based on the clinical presentation, the related clinical features, and documented absence of other potentially confounding diagnoses, including ruling out CCHS with clinically available *PHOX2B* testing (documenting an absence of PARMs and NPARMs).

Mosaicism in a Subset of Parents with CCHS Children

Most expansion mutations occur *de novo* in CCHS, but 5 to 10% are inherited from a mosaic typically unaffected parent. A distinction is needed between germline inheritance and somatic occurrence of the *PHOX2B* mutation. Incomplete penetrance has been demonstrated when certain *PHOX2B* mutations are present in all the cells (including the reproductive cells of the germline) of individuals known to be unaffected. These latter *PHOX2B* mutations (20/24, 20/25, and a few NPARMs), although asymptomatic in some individuals, may be characterized by milder or variable phenotypic effects in the children affected with CCHS or other family members (3, 5, 17), respectively. In contrast, somatic mosaicism, due to postzygotic mutations, has been reported among a subset of parents of typical CCHS cases carrying *PHOX2B* polyalanine (PA) alleles larger than 25 repeats and NPARMs (3, 6, 7, 16).

Somatic mosaicism for a PARM was first reported in 2003 by Weese-Mayer and colleagues (3) in 4 parents out of 54 available families (7.4%). In 2005 Trochet and colleagues (6) identified somatic mosaicism in 1 parent of each of 10 CCHS patients, confirming that roughly 10% of children with CCHS will inherit the mutation from a mosaic parent. In both studies, mosaicism was detected in DNA extracted from parents' peripheral leukocytes by observing a lighter signal from the expanded

allele than from the normal allele, in contrast to the pattern seen in subjects with CCHS.

A quantitative estimate of the somatic mosaicism in unaffected parents has recently been assessed in two other studies. DNA amplification products from asymptomatic carriers of alanine expansions with genotypes ranging from 20/25 to 20/31 were loaded on a DNA automated sequencer and expanded, and normal alleles were visualized (Figure 5) as output peaks whose underlying area was directly proportional to their respective amounts. Although the mutant peak was expected to represent 50% of the *PHOX2B* alleles in individuals who inherited the mutation, it was found to range from 9 to 35% in DNA from parental leukocytes; this percentage was confirmed in studies of fibroblast and saliva DNA from a subset of these mosaic parents (13). Likewise, in another study, mosaic individuals were identified as "outliers," with less signal in the peak corresponding to the expanded allele (16). Whereas somatic mosaicism for PARMs larger than 20/25 was demonstrated in these studies, none of the rare seemingly asymptomatic 20/25 carriers were found to be mosaic, confirming that in these cases lack of the disease phenotype can be ascribed to reduced penetrance of a germline mutation (13, 16). Taken together, these data support the hypothesis that germline PARMs larger than the 20/25 genotype are fully penetrant, and asymptomatic carriers may only be found in association with significant degrees of somatic mosaicism. The CCHS phenotype has not been associated with any degree of somatic mosaicism thus far, suggesting a germline origin for most PARMs in affected CCHS patients.

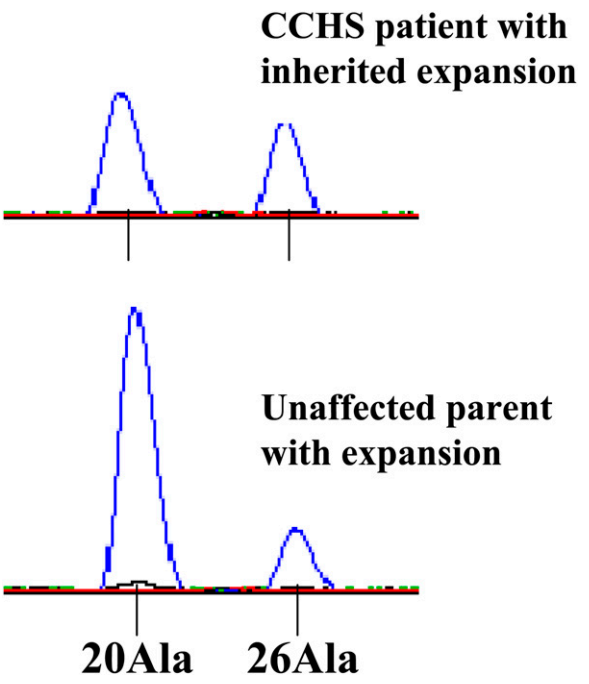

Figure 5. Differential amounts of wild-type and expanded *PHOX2B* alleles in PA mutation carriers. *Blue* peaks represent the *PHOX2B* alleles carried by a patient with congenital central hypoventilation syndrome (CCHS) (*upper*) and an asymptomatic parent (*bottom*). Although positions in the x-axis correspond to their lengths, as indicated underneath, peak height is directly proportional to their amount. In this light, the individual below presents a somatic mosaicism for the 26 Ala allele (genotype 20/26), which in fact correspond to much less than half with respect to the 20 Ala wild-type allele (normal genotype 20/20).

Inheritance of CCHS and the *PHOX2B* Mutation

Detection of the same *PHOX2B* mutation in parent-child pairs and observation of somatic mosaicism in some unaffected parents for the mutation observed in their affected child clearly established an autosomal dominant inheritance pattern for CCHS (3, 6). Most parents of affected children with CCHS do not carry a mutation at all, indicating a high *de novo* mutation rate in affected individuals. The 20/24 and 20/25 genotype PARMs and some of the NPARMs may be found in the germline of asymptomatic parents of children with CCHS and even other family members, suggesting these mutations are inherited as dominant with incomplete penetrance (5, 7, 10, 11, 13, 17, 26). Family members who carry such mutations but do not have CCHS may show other ANSD phenotypes, including HSCR or neuroblastoma (7, 48), or may be presymptomatic, presenting in later childhood or adulthood.

Genetic counseling is crucial for individuals diagnosed with CCHS, their parents and, in some cases, specific family members. For all affected individuals with CCHS, there is a 50% chance of transmitting the mutation, and therefore the disease phenotype, to each offspring. If an unaffected parent is found to be mosaic for a *PHOX2B* mutation (usually identified because of an affected child), there will be up to a 50% chance of recurrence in any subsequent child. Mosaic individuals always can be assumed to have a new mutation (the mutation cannot be inherited in mosaic fashion) and therefore, only children of these individuals (not other family members) would be at risk to have the mutation. If unaffected parents do carry a germline mutation (i.e., a 20/25 genotype PARM) there may be numerous other family members who can carry the same mutation without having obvious symptoms. In this case, genetic testing is indicated for all persons in position in the pedigree to inherit the mutation, which can often be traced back until the individual in whom the mutation originated is identified. To assess recurrence risk in a family, both parents of children with CCHS should have the *PHOX2B* Screening Test done to rule out mosaicism (90) (PARMs of genotypes 20/26 to 20/33 and severe NPARMs) or a nonpenetrant carrier state (genotypes 20/24 and 20/25 PARMs and mild NPARMs). Prenatal testing is available and can be performed for individuals with or without CCHS who are known germline mutation carriers or recognized as somatic mosaics. Despite negative testing of parents of a CCHS child, germline mosaicism cannot be ruled out and prenatal testing for subsequent pregnancies should be considered. Prenatal testing can allow parents optimal information with which to make an informed decision with a range of possibilities from elective abortion to a fully prepared delivery room to optimize the baby's chance for a smooth transition to extrauterine life.

Mechanism of PARMs

Polyalanine tracts, predicted in roughly 500 human proteins, are preferentially found in transcription factors and are regarded as flexible spacer elements essential to conformation, protein–protein interactions, and/or DNA binding. PA tracts coded by human genes, other than *PHOX2B*, have already been found expanded in association with at least nine different congenital disorders, including mental retardation and malformations of the brain, digits, and midline structures (91). In this light, PA expansions are members of a broader category of trinucleotide repeat–associated disorders that includes also polyglutamine (PQ) expansions. Unlike PQ tracts, PA stretches are generally stable (do not change size when passed on from one generation to another), are usually coded by imperfect trinucleotide re-

peats (alanine can be coded by four different DNA triplets) and, with the exception of rare contractions, are not present as polymorphic tracts in the human population (most wild-type genes all have the same number of alanines). These observations have suggested an unequal allelic homologous recombination (crossover) during meiosis and/or mitosis as the most attractive disease-causing mechanism for poly-A tract expansions (91).

However, in mosaic individuals, only two alleles (wild-type and expanded alleles), instead of the three alleles (wild-type, contracted, and expanded) expected after occurrence of a somatic event of unequal crossing-over, have been reported, demonstrating that an alternative mutational mechanism should be considered to explain the origin of these trinucleotide repeat expansions (16, 92). Indeed, by reasoning that imperfect trinucleotide repeat sequences, typical of PA tracts, would reduce the ability of the repeats to form misaligned structures, replication slippage has been proposed as a more plausible mechanism than unequal crossing over for the generation of PA expansions (93).

This view has recently been revised after observing four families informative for *PHOX2B* markers whose segregation was compatible with the occurrence of unequal sister chromatid exchange. It is probable that *de novo* expansion of PA repeats in CCHS results mainly from this sort of chromosomal event either during gametogenesis or in postzygotic somatic cells (94).

A paternal origin of the gametes transmitting expansions has been reported in six informative *de novo* CCHS trios (94), whereas in a larger cohort of 20 trios, 13 mutations occurred on the paternal and 7 on the maternal chromosomes. Thus, occurrence of PA repeat expansions may be independent from processes specific to sperm or oocyte development, or it may be that there is a weak gender bias that would require analysis of a larger sample of parent-child trios.

Mechanism by Which Mutations in the *PHOX2B* Gene Result in the CCHS Phenotype

As a tissue-specific transcription factor, *PHOX2B* is responsible for the expression regulation of a series of target genes involved in the development of the ANS. The finding that PHOX2B binds directly to the regulatory regions of the dopamine-β-hydroxylase (*DβH*), *PHOX2A*, and *TLX-2* genes has allowed application of a functional approach to disclose the molecular mechanisms underlying CCHS pathogenesis. To this end, *PHOX2B* mutations have been tested for potential disruption of the normal function of the protein with respect to (*i*) transactivation of different target promoters, (*ii*) DNA binding, (*iii*) aggregate formation, and (*iv*) subcellular localization. Distinct CCHS pathogenetic mechanisms for PARMs and NPARMs in *PHOX2B* have thus been postulated. In addition, the cellular response to *PHOX2B* polyalanine expansions has been investigated to determine whether there exist cellular mechanisms that could be targeted to limit the cytotoxicity of these mutations.

PHOX2B PA mutations (PARMs). To investigate how *PHOX2B* PA expansions can induce CCHS pathogenesis, the ability of expression constructs containing PA mutations to regulate the transcription of known target genes has been compared, in two different laboratories, to a wild-type *PHOX2B* construct. In particular, as exemplified in Figure 6A for the DβH target gene, when mutant *PHOX2B* constructs were cotransfected with the DβH and *PHOX2A* regulatory regions connected to the *Luciferase* gene, a strict inverse correlation between the induced Luciferase activity and the length of the PA tract was identified. This suggested that the transcriptional regulation of these two genes is directly dependent on the correct structure of the PHOX2B domain, including the 20-alanine tract and that longer tracts increasingly disrupt transcription (6, 18). Finally, a significant reduction of the transactivating activity of PHOX2B constructs bearing different PA contractions on the DβH promoter has also been observed in the same reporter assay (72). Unfortunately, this observation could not be replicated in another cell recipient (6) suggesting the need for additional investigation before assessing whether, despite lack of any phenotypic effect, PA contractions result in some disruption of *PHOX2B* function.

Fluorescence microscopy of COS-7 cells expressing PHOX2B proteins fused to a green fluorescent molecule has shown that the wild-type PHOX2B protein is present almost exclusively in the nucleus. However, increasing length of the PA repeat does induce an increasing percentage of PHOX2B protein within cells to mislocalize to the cytoplasm (Figure 7) (18). As such, similar experiments performed in HeLa cells induced formation of PHOX2B polyalanine aggregates, although in different amounts compared with that observed in COS-7 cells (6), suggesting that mislocalization of the mutant protein is a common pathogenetic mechanism leading to impaired transcriptional activity of mutant PHOX2B containing aggregation-prone expanded PA tracts.

Moreover, based on electrophoretic mobility shift assays, it has been observed that expansions containing 29 alanines and more do affect PHOX2B DNA binding, probably because aggregated PHOX2B mutant proteins are not available for DNA binding, an observation confirmed *in vitro* by showing that PA expanded PHOX2B proteins spontaneously form oligomers (6). Finally, the interaction between the wild-type PHOX2B protein with the misfolded 33 repeats mutant has suggested that PA mutations can also prevent the normal protein from its usual function because of abnormal aggregation with the mutant (6, 18).

In the attempt to assess the fate of cells expressing PA-expanded *PHOX2B*, *in vitro* experiments have demonstrated that activation of the heat-shock response by the drug geldanamycin, a naturally occurring antibiotic, is efficient both in preventing formation and in inducing the clearance of PHOX2B preformed PA aggregates and, ultimately, also in rescuing the PHOX2B ability to transactivate the DβH promoter. In addition, elimination of PHOX2B mutant proteins by the proteasome and autophagy, two cellular mechanisms already known to be involved in the clearance of proteins containing expanded polyglutamine and polyalanine tracts, has been demonstrated. Cellular apoptosis has been observed only in association with the largest PA expansions (19).

PHOX2B non-PA mutations (NPARMs). Non-PA mutant PHOX2B proteins tested so far have shown compromised transcriptional activation of the DβH and TLX2 promoters with more severe activity disruption correlated with length of the frameshifted C-terminal sequence (*see* Figure 6B for the effect on the DβH target) (6, 18, 95). Unexpectedly, *PHOX2B* frameshift mutations have shown a 10 to 30% increased activation of the *PHOX2A* regulatory region (18). Moreover, frameshifts and missense mutations have mainly shown a complete loss of DNA binding but, unlike the long PA expansions, are able to correctly localize in the nucleus (Figure 7) (6, 18).

Aberrant C-terminal regions may cause PHOX2B protein dysfunction due to either lack of ability to establish correct protein–protein interactions with molecular partners or gaining of the ability to interact with wrong molecules, a very attractive hypothesis in light of the association of NPARM mutations with risk of neuroblastoma development (96). Consistently, a recent study has shown that non-PA mutant *PHOX2B* constructs

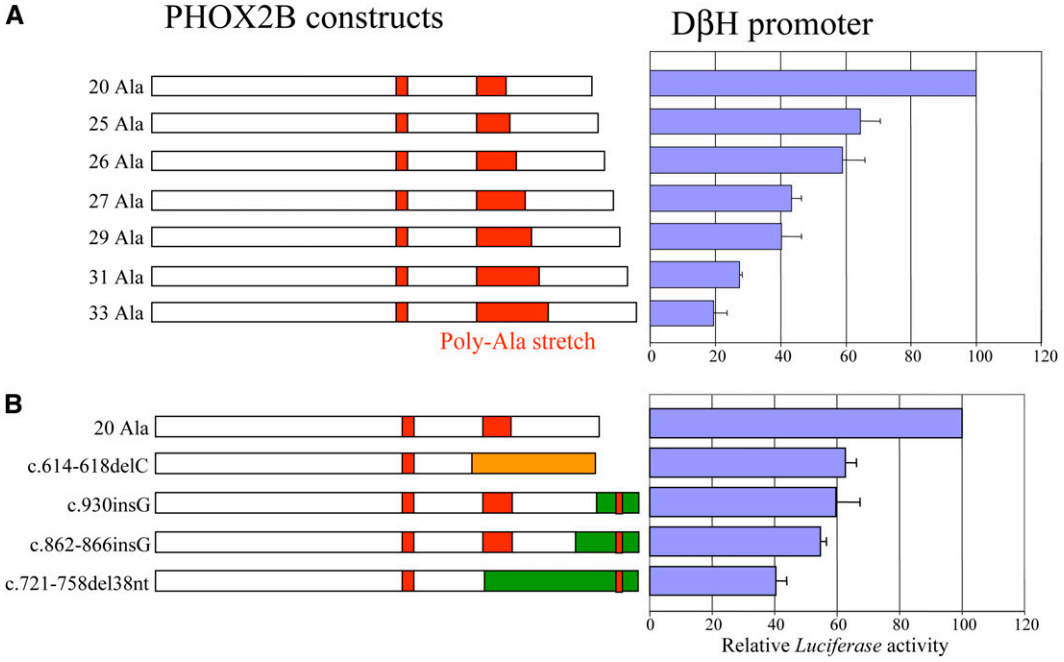

Figure 6. Effect of *PHOX2B* mutations on transactivation of the *DβH* regulatory region. The transcriptional activity obtained by cotransfecting the *PHOX2B* expression constructs reported on the left of each diagram with a construct containing the *DβH* promoter cloned upstream of the *Luciferase* reporter gene is shown for both (*A*) poly-Ala expanded tracts and (*B*) frameshift mutations in terms of relative Luciferase activity. Adapted by permission from Reference 18.

retained the ability to suppress cellular proliferation without being able to promote differentiation (20), suggesting a mechanism that might promote the development of neural crest tumors.

In conclusion, these studies have indicated a marked difference between PARMs and frameshift NPARMs in terms of transactivation of target promoters, formation of aggregates, and subcellular localization. Future *in vitro* investigation will provide further clues on pathogenesis and possible therapeutic hints.

Clinical Aspects of CCHS

Diagnosis and clinical course. Before the 2003 discovery that *PHOX2B* is the disease-defining gene for CCHS, the clinical spectrum of severity in terms of hypoventilation and other aspects of the ANSD phenotype had long puzzled clinicians. Once the diagnosis of CCHS is considered, blood should be sent for the *PHOX2B* Screening Test (*see* Figure 8). In the event the screening test is negative, and the patient's phenotype supports the diagnosis of CCHS or LO–CCHS or the physician/family wants to completely rule out the diagnosis of CCHS, then the sequel *PHOX2B* Sequencing Test should be performed (available at Children's Memorial Hospital, Chicago, IL; for additional options please refer to www.genetests.org). This two-step testing is most cost efficient (the mutation in 95% of CCHS cases will be identified with the inexpensive *PHOX2B* Screening Test and only a subset of the NPARMs will require the *PHOX2B* Sequencing Test to be identified). While awaiting results of the clinically available *PHOX2B* testing (high sensitivity and specificity) other causes of hypoventilation should be ruled out to expedite proper intervention and facilitate treatment strategies for home care. Primary lung disease, ventilatory muscle weakness, and cardiac disease should be ruled out with the following tests: chest x-ray and potentially chest CT, comprehensive neurological evaluation and potentially muscle biopsy, and echocardiogram, respectively. Causative gross anatomic brain/brainstem lesions should be ruled out with an MRI and/or CT scan of the brain and brainstem (97, 98). Likewise, inborn errors of metabolism should be considered, and a metabolic screen should be performed.

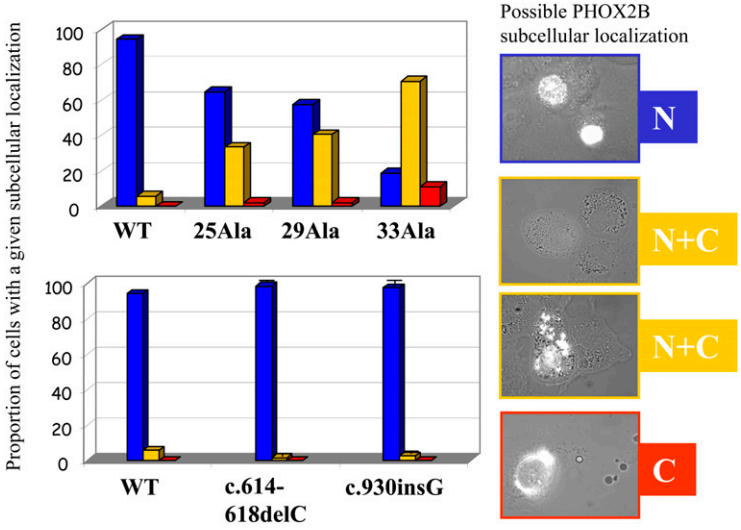

Figure 7. Subcellular localization of proteins bearing different PHOX2B defects. In the panels on the right, three possible patterns of PHOX2B cellular localization are reported: (*N*) nuclear localization only; (*N*+*C*) both nuclear and cytoplasmic localization, either diffuse or with formation of nuclear and/or cytoplasmic aggregates; and (*C*) cytoplasmic localization only. Subcellular distribution of PHOX2B-GFP proteins, after cell-transfection with mutant constructs carrying different PHOX2B mutations, is reported in the histograms on the left: 25Ala, 29Ala, 33Ala (*above*) and c.930insG, c.614–618delC (*below*). Adapted by permission from Reference 18.

Independent of respiratory control abnormalities, children with CCHS have other evidence for diffuse autonomic dysregulation (27, 28). For those individuals with constipation symptoms, a barium enema or manometry and potentially full thickness rectal biopsy should be performed to diagnose HSCR (99). Serial chest and abdominal imaging is essential among children with the NPARMs and those children with the 20/29 to the 20/33 genotype for emergence of a neural crest tumor, specifically neuroblastoma (NPARMs) and ganglioneuroblastoma/ganglioneuroma (PARMs) (100). Because no children with genotypes 20/24 to 20/28 have been identified with tumors of neural crest origin, the value of serial imaging in these cases is unknown. Cardiac rhythm abnormalities, including decreased beat-to-beat heart rate variability, reduced respiratory sinus arrhythmia, and transient abrupt asystoles, have been described (9, 101, 102). Seventy-two–hour Holter monitoring performed annually may determine aberrant cardiac rhythms, sinus pauses that will necessitate bipolar cardiac pacemaker implantation (103), and the frequency of shorter pauses (i.e., less than 3 s) that may have physiologic and neurocognitive impact. Children with CCHS are at risk for progressive pulmonary hypertension and cor pulmonale as a result of recurrent hypoxemia due to inadequate ventilator settings or tracheostomy caliber, unrecognized hypoventilation during spontaneous breathing while awake, excessive exercise with resultant physiologic compromise, or suboptimal compliance with artificial ventilation. As a result, echocardiograms, hematocrits, and reticulocyte counts performed every 12 months will provide information regarding potential cor pulmonale and polycythemia, with testing performed more frequently if clinically indicated and warranted. CCHS patients frequently exhibit ophthalmologic abnormalities reflecting the role of *PHOX2B* on the cranial nerves controlling pupillary function (23, 29). Comprehensive ophthalmologic testing will determine the nature of the ophthalmologic involvement and allow for intervention strategies to avoid interference with learning. Anecdotal reports of poor heat tolerance and profuse sweating have been described (1) but not studied comprehensively. Very limited formal assessment of the ANS has been reported, and none have been analyzed by *PHOX2B* genotype. Comprehensive autonomic testing as clinically indicated to assess syncope and to assess autonomic nervous system function may include tilt testing, deep breathing, Valsalva maneuver, thermal stressors, pupillometry, and more, as new measures of autonomic testing are developed for infants and children.

Suboptimal school performance and/or decreased intellectual function have been observed in CCHS patients (23, 104–107). It is unclear whether this is due to hypoxemia from inadequate ventilatory support or a direct result of the primary neurologic problem associated with CCHS. As children with CCHS are more consistently identified in the newborn period, and as management for these complex and vulnerable children becomes more standardized, improved neurocognitive performance is anticipated with distinction between sequelae of hypoxemia (due to hypoventilation or asystoles) and innate disease specific to CCHS. Comprehensive neurocognitive testing performed annually in a controlled setting will assess the child's progress relative to intervention, management, and compliance and may identify areas for intervention. Children with CCHS have a good future with the oldest neonatally identified patients graduating from college, getting married, and maintaining employment. It behooves the family and medical personnel to provide optimal oxygenation and ventilation to assure maximization of neurocognitive potential. Aggressive educational intervention coupled with careful ventilatory and cardiovascular management is essential (23, 104–107).

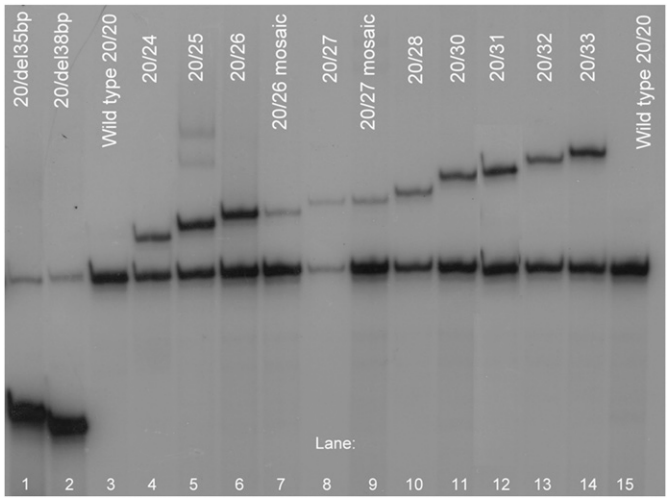

Figure 8. Polyacrylamide gel electrophoresis *PHOX2B* Screening Test. Shown are *PHOX2B* polyalanine repeat expansion mutations (PARMs) by polyalanine repeat size and non-PARMs (NPARMs) for the most common congenital central hypoventilation syndrome (CCHS)-causing *PHOX2B* genotypes identified with the *PHOX2B* Screening Test compared with the wild-type (lanes 3 and 15) product. Lanes 1–2 indicate results for NPARMs with large deletions: 722del35 and 722del38. Lanes 4–14 indicate analysis of several PARMs, including mosaic parents (genotype 20/26 and 20/27) as well as probands (genotypes 20/24–20/33). Lanes 7 and 9 are mosaic carriers of CCHS-causing PARMs. Lane 7 is a mosaic parent of the proband identified in lane 6. Lane 9 is a mosaic parent of the proband identified in lane 8. Note that the band intensity of the expanded allele is much lighter than that of the wild-type allele in lanes 7 and 9. Also note that although the overall intensity of the signal in lane 8 is low, both the expanded and wild-type bands are similar in intensity indicating a full carrier of the expanded 27 repeat allele. Reproduced by permission from Reference 41.

In the ideal situation, care of individuals with CCHS would be provided through centers with extensive expertise in CCHS, working in close partnership with regional pediatric pulmonologists and pediatricians. That arrangement will further improve the consistency of management and ideally improve the level of outcome for individuals with CCHS. Although there may be resistance to referral to such centers, the ATS Statement authors note that many exceedingly capable pediatric pulmonologists are basically managing children with CCHS as they would other patients with tracheostomies and ventilators. They are seeing the patients at varying intervals ranging from 4 to 12 months, only checking physiologic measures for 5 minutes awake in the clinic, not taking an autonomic medicine history, not reviewing the technology in the home or access to emergency power and care, not reassessing tracheostomy tube size after the neonatal period, not changing from neonatal ventilator tubing to pediatric tubing, not educating parents in ventilator management and usefulness of noninvasive monitoring, not advocating that all parents be screened with the *PHOX2B* Screening Test to ascertain mosaicism, and more. Essentially they are extremely capable but busy practitioners who do not have the time or *intensive* experience to focus on the nuances that are essential to the successful management of children with CCHS and their families. The aim is to accept that caring for children with CCHS is a privilege that requires a depth of care not typical for other patients with more common pulmonary disorders. There is simply not enough time for the pediatric pulmonologist (who is juggling patients with cystic fibrosis,

chronic lung disease, asthma, and more) to provide in-depth attention to the needs of a child with CCHS. Because of the nature of the *PHOX2B* mutations, and the range of phenotype based on these mutations, experience with even 15 to 30 patients does not begin to provide the scope of experience necessary for understanding the needs of the child with CCHS.

Hypoventilation and incidence. Alveolar hypoventilation is the hallmark of CCHS, and its most apparent and potentially debilitating phenotypic feature. Characteristically, the diminution of tidal volume with resultant effect on minute ventilation is most apparent in non–REM sleep in CCHS, but it is also abnormal during REM sleep and wakefulness, although usually to a milder degree (23, 108, 109). The spectrum of sleep-disordered breathing may range in severity from hypoventilation during non–REM sleep with adequate ventilation during wakefulness, to complete apnea during sleep and severe hypoventilation during wakefulness. The CCHS phenotype relative to ventilatory needs is *PHOX2B* genotype/mutation-dependent. Typically, children with the 20/27 to 20/33 genotype and the NPARMs will require 24 hours per day of mechanical ventilation. Children with the 20/24 and 20/25 genotypes, and a small subset of NPARMs, rarely require 24 hours per day of ventilation unless they have had suboptimal ventilatory management for prolonged periods in early childhood. The awake ventilatory needs of the children with the 20/26 phenotype will vary with the activity level. It remains unclear whether spontaneous breathing while awake will improve with puberty.

The incidence of CCHS in the general population is unknown, although the incidence will likely vary by ethnicity based on current statistics (~90% of identified subjects are Caucasian in review of published reports and in personal communication from the laboratories of the members of the *ad hoc* Statement Committee). With recognition that individuals with the 20/24 and 20/25 genotypes have variable expressivity, and might not present to medical attention until they receive sedation or have severe pulmonary illness, it behooves the medical community to identify such patients before being faced with a life-threatening situation. What is apparent is that the incidence of CCHS is no longer as rare as anticipated at the time of the first ATS Statement on CCHS in 1999 (1). Determination of a true incidence will require a large population-based study across all ethnic groups.

Respiratory physiologic perturbation. The results from reports of awake and asleep ventilatory and arousal responses (108, 110–112), mental concentration (34), respiratory sensations (35, 36), physiologic response to exercise and leg motion (32–35, 113, 114), and focal abnormalities on functional MRI (115–118) must be interpreted with caution as they likely reflect bias due to small sample size, reporting in the pre-*PHOX2B* era, data presentation inclusive of subjects with CCHS and with other causes of hypoventilation (108), or without documentation of specific *PHOX2B* confirmation of CCHS in all subjects, and near-exclusive inclusion of children who were able to sustain adequate ventilation during wakefulness at rest (so likely nearly all subjects had the 20/25 genotype based on the described phenotype and protocols). Until the studies are repeated in large cohorts including a broad array of children and adults with genotypes 20/24 to 20/33 as well as the NPARMs, these results may represent the physiology of only the mildest patients with CCHS.

Biannual then annual in-hospital comprehensive physiologic studies during awake and asleep states to assess ventilatory needs during varying levels of activity and concentration, in all stages of sleep, with spontaneous breathing and with artificial ventilation, and ventilatory responsivity to endogenous and exogenous physiologic challenges awake and asleep, will ascertain each child's needs for optimal clinical management. These studies, performed over the course of a several-day hospitalization in a center with extensive CCHS experience, will allow for a clear understanding of needs when breathing spontaneously as well as with artificial ventilation by any of the means described in the sections that follow. These physiologic studies should include constant supervision by highly trained personnel and continuous audiovisual surveillance with continuous recording (at a minimum) of respiratory inductance plethysmography (chest, abdomen, sum), ECG, hemoglobin saturation, pulse waveform, end tidal carbon dioxide, sleep state staging, blood pressure, and temperature. Other recommended testing for individuals with *PHOX2B* mutation-confirmed CCHS is provided in Table 1.

Ventilatory Management

The primary goals are to secure the airway and ensure optimal ventilation and oxygenation with artificial ventilation. As CCHS does not resolve spontaneously, nor does it appear to respond to pharmacologic stimulants (1, 22, 23, 119) or improve with advancing age except in rare anecdotal cases, chronic ventilatory support at home is necessary for these patients to leave the hospital and grow up with their families. Positive pressure ventilators via tracheostomy (1, 120–122), bilevel positive airway pressure (1, 123–131), negative pressure ventilators (132, 133), or diaphragm pacing (134–145) are options for these patients. Although oxygen administration without artificial ventilation improves the Pa_{O_2} and relieves cyanosis, this treatment is inadequate as hypoventilation persists and pulmonary hypertension ensues.

Until internally implanted biofeedback ventilatory support via electrical stimulation is developed, the aim is to compensate for the altered or absent ventilatory responses to hypoxemia and hypercarbia during awake and asleep states in individuals with CCHS. It cannot be overemphasized that patients with CCHS may suffer complete respiratory arrest or severe hypoventilation at sleep onset. They therefore require continuous observation and/or monitoring so that ventilatory support can be initiated with each sleep episode and ideally before sleep onset. Apnea/bradycardia transthoracic impedance monitoring will not detect the hypoventilation experienced by children with CCHS, nor will it detect tracheal obstruction, as the technique does not determine obstructed breaths (146). Likewise, these monitors will not detect the sinus pauses characteristic of children with CCHS as they are abrupt and may spontaneously terminate before the averaging algorithm in the monitor detects the events. Furthermore, for those children who sleep with diaphragm pacers, the pacer artifact will add to the number of recorded heart beats (146) thereby falsely elevating the heart rate. Thus, there is no role for an apnea/bradycardia monitor in caring for the child with CCHS. Healthy normal children will characteristically respond to hypoxemia and/or hypercarbia with increased work of breathing (retractions, deeper breaths, increased respiratory frequency) and a sense of shortness of breath. However, children with CCHS lack these characteristic clues, so that clinicians must rely on objective measures of oxygenation and ventilation (pulse oximeter and $P_{ET_{CO_2}}$ monitor used continuously in the home during all sleep time and ideally for hourly/periodic checks during awake time) as well as continuous care by a registered nurse trained and experienced in ventilator management to prevent significant and sustained hypoxia and its physiologic and neurocognitive sequelae. Pulse oximeter and end tidal carbon dioxide monitors are usually set

TABLE 1. RECOMMENDED TESTING TO CHARACTERIZE CCHS PHENOTYPE

PHOX2B Genotype	Annual In-Hospital Comprehensive Physiologic Testing (Awake and Asleep), Exogenous and Endogenous Gas Challenges, Autonomic Testing*	Assessment for Hirschsprung Disease	Annual Neurocognitive Assessment*	Annual 72-h Holter Recording and Echocardiogram*	Annual Imaging to Assess for Tumors of Neural Crest Origin
PARM					
20/24 and 20/25	X		X	X	
20/26	X	X	X	X	
20/27	X	X	X	X	
20/28–20/33	X	X	X	X	X†
NPARM	X	X	X	X	X‡

Definition of abbreviations: PARM = polyalanine repeat expansion mutation; NPARM = nonpolyalanine repeat expansion mutation (missense, nonsense, frameshift).

* Infants under the age of three years should undergo comprehensive evaluations every 6 months.

† Annual chest and abdominal imaging to identify ganglioneuromas and ganglioneuroblastomas.

‡ Abdominal imaging and urine cathecholamines every 3 months in first 2 years, then every 6 months until 7 years of age to identify neuroblastomas.

to alarm for Sp_{O_2} 85% or less and $P_{ET_{CO_2}}$ of 55 mm Hg or greater, respectively. Note that the alarm thresholds are different than the desired levels of Sp_{O_2} and $P_{ET_{CO_2}}$ to reduce nuisance alarms but still give caregivers adequate time to respond to potential emergencies. Because the innate mechanisms of respiratory drive are absent/attenuated in CCHS, without the noninvasive monitoring and the continuous supervision, children with CCHS may experience profound hypoxemia with related sequelae before the physiologic compromise is detected. Central respiratory drive can be further inhibited by metabolic imbalance, such as chronic metabolic alkalosis. Sedative medications and central nervous system depressants should be avoided as much as possible, as they worsen the hypoventilation. The child with CCHS should ideally not be swimming. However, if swimming, they should be carefully supervised regardless of the presence or absence of a tracheostomy. Children with CCHS should be prohibited from competing in underwater swimming contests as they will not perceive the asphyxia that occurs with drowning and breath-holding and therefore will swim longer and farther than children without CCHS—thereby heightening the risk of drowning.

Selecting the best mode of artificial ventilatory support for each individual. Although infants and children with CCHS have occasionally been managed exclusively with noninvasive ventilation, this is not considered to be the optimal form of management. To ensure optimal oxygenation and ventilation beginning in the first days of life, and to provide for optimal neurocognitive outcome, the authors of this ATS Statement recommend positive pressure ventilation via tracheostomy in the first several years of life. A power generator in the event of a power outage or natural disaster as well as placement on the emergency list for the local power company and fire department will assure the family of access to immediate care. Noninvasive ventilation is not a consideration in conservative management until 6 to 8 years of age at the earliest in stable patients with CCHS requiring ventilatory support only during sleep.

Philosophy of chronic ventilatory support. Weaning from the ventilator is not a realistic goal and should not be considered in CCHS. Children with CCHS cannot be "trained" to breathe adequately, either. To optimize quality of life, these children need available energy for other academic and physical activities. Thus, ventilators and pacers are adjusted to completely meet the ventilatory demands of these children, with settings specific to varying levels of activity and leaving much of the child's energy available for other activities. A reasonable range is $P_{ET_{CO_2}}$ 30 to 50 mm Hg (although ideally 35–40 mm Hg) and Sp_{O_2} of 95% or higher. Some centers use "ventilator ladders" in conjunction with pulse oximetry and $P_{ET_{CO_2}}$ monitoring to maintain precise control of gas exchange within a narrow normal range. It is important to maintain normal oxygenation to avoid risk for deficits in cognition.

Modalities of chronic ventilatory support. Because children with CCHS usually do not have lung disease, they have the greatest number of options for different techniques to provide chronic artificial ventilation at home. These include: (*1*) portable positive pressure ventilator via tracheostomy (1, 120–122); (*2*) bilevel positive airway pressure via nasal or face mask (123–131); (*3*) negative pressure chest shell (cuirass), wrap, or portable tank ventilator (132, 133); or (*4*) diaphragm pacing (134–145).

Portable positive pressure ventilator via tracheostomy. The portable positive pressure ventilator is the most common method of providing home mechanical ventilation in CCHS (23, 147–150). Commercially available electronic home positive pressure ventilators have battery capability and are relatively portable. A tracheostomy is required for positive pressure ventilator access. A tracheostomy tube smaller than the airway caliber may reduce the likelihood of tracheomalacia, allow for a leak adequate to use the Passy-Muir one-way speaking valve while off the ventilator, and allow for a margin of safety if the tube becomes occluded. By using such speaking valves, children with CCHS learn to speak relatively normally from early childhood onward. However, if the tracheostomy is too small, nocturnal mechanical ventilation will be inadequate due to the air leak, and a cuffed tracheostomy tube may need to be considered. Thus, determinations of adequacy of the tracheostomy size at initial management and with subsequent growth should be made during annual inpatient physiologic evaluation at a center with extensive experience in the care of children with CCHS.

Using the home ventilator in a pressure plateau mode or pressure control mode may compensate for a small leak, although the use of a tight-to-the-shaft cuffed tracheostomy tube may be necessary with some home ventilators. If the same peak inspiratory pressure is achieved on each breath, the lungs are inflated to the same tidal volume, dependent on pulmonary mechanics, regardless of the amount of leak around the tracheostomy. Because of this, pressure ventilation is preferred, and it can be easily used on portable home ventilators with continuous flow. Ideally, a second ventilator in the home for those individuals with CCHS who rely on mechanical ventilation, regardless of the duration of hours of use each day, may prevent emergency admission in the event of ventilator failure.

Tracheostomy. Since the 2003 introduction of clinical *PHOX2B* testing to confirm the diagnosis of CCHS, children are now definitively diagnosed within the first weeks of life and the tracheostomy is typically performed before 1 month of age. As the patient grows, the tracheostomy tube must be upsized to provide adequate ventilation. Signs that a tracheostomy tube size may need to be increased are sometimes subtle but include difficulty achieving adequate gas exchange and a visible plateau on $P_{ET_{CO_2}}$ monitoring, having to increase ventilator settings to levels above those of other similar-aged children, more frequent

pneumonias, and an audible air leak. A spare tracheostomy tube is necessary as a back-up at all times and should be carried with the Ambu bag for any travel outside of the home. Bronchoscopy performed by an experienced pediatric otolaryngologist every 12 to 24 months will allow for early diagnosis of granulomas or other airway abnormalities.

Ventilator circuits. A circuit is required to deliver air from a positive pressure ventilator to the patient through a heated humidification system (essential for infants and children; the desired temperature range is 26–29°C, 80–85°F) connected to the tracheostomy with a swivel adapter. Dead space between the tracheostomy and exhalation valve should be minimized, to avoid elevated P_{CO_2} due to rebreathing. Two to three circuits are generally provided for home care, and they are changed each day. The circuits should be appropriate for the weight of the child.

Bilevel positive airway pressure ventilation by mask or nasal prongs. Noninvasive intermittent positive pressure ventilation is delivered via a nasal mask, nasal prongs, or face mask using a bilevel positive airway pressure ventilator, although the full face mask is discouraged because of patient discomfort and concerns for vomiting/aspiration. Bilevel ventilators are smaller, less expensive, and generally easier to use than conventional ventilators, but they are not designed for life support. They provide variable continuous flow via a blower (fan), have a fixed leak (that prevents CO_2 retention), and can compensate for leaks around the mask. Inspiratory positive airway pressure and expiratory positive airway pressure can be adjusted independently (difference is proportional to tidal volume), maintaining a large inspiratory positive airway pressure to expiratory positive airway pressure difference as tidal volume increases linearly, up to approximately 14 cm H_2O. Only the timed mode guarantees breath delivery in children who cannot generate adequate large spontaneous breaths to trigger the ventilator. Bilevel ventilation should not be used outside of sleep time as the mask interferes with daily activities and social interaction, and the risk of skin breakdown increases.

Because mask ventilation has been associated with mid-face hypoplasia when introduced from infancy or early childhood, mask ventilation should be used with extreme caution in young children with a malleable mid-face, which may be more prone to compression and deformation by a tight-fitting face or nasal mask. A pediatric plastic surgeon and orthodontist/oral surgeon should closely follow any child using mask ventilation. Children may require intubation and more sophisticated ventilatory support during acute respiratory illnesses. The major benefit of bilevel ventilation is that a tracheostomy is not required, although successful management with bilevel ventilation is most effective in older children and adults with the milder CCHS phenotypes. Bilevel positive airway pressure ventilation should not be used with a tracheostomy. If a child has a tracheostomy, ventilation is much more reliable and effective using a positive pressure ventilator than using bilevel ventilation. A limited number of children with CCHS have been successfully transitioned from positive pressure ventilation via tracheostomy to bilevel positive airway pressure ventilation by mask or nasal prongs after 6 to 8 years of age.

Negative pressure ventilation. Negative pressure ventilators apply a negative pressure outside the chest and abdomen with the chest shell, wrap, or portable tank to expand the chest and upper abdomen. These ventilators can provide effective ventilation in children and adolescents sometimes without a tracheostomy. However, many infants and young children will require a tracheostomy, thereby mitigating the potential benefit of the negative pressure ventilation. Additional limitations include portability (not battery operated), difficulty sleeping in the supine position, skin irritation, and a sense of feeling chilled. A limited number of children with CCHS have been successfully transitioned from positive pressure ventilation via tracheostomy to negative pressure ventilation without tracheostomy after 6 to 8 years of age.

Diaphragm pacing. Diaphragm pacing generates breathing using the child's own diaphragm as the respiratory pump (134–145). The battery-operated external transmitter generates a train of pulses that are transmitted via an external antenna. The antennae create a radio frequency signal that is communicated to the subcutaneously implanted receivers bilaterally. The subcutaneously implanted receivers convert the radio frequency signal into an electrical current that is transmitted via stainless steel wires connecting the receivers to the monopolar platinum phrenic nerve electrodes. The electrical stimulation of the phrenic nerve causes a diaphragmatic contraction, which generates the breath. Bilateral implantation of phrenic nerve electrodes and diaphragm pacer receivers is recommended to achieve optimal ventilation in children. In carefully identified patients (no or mild intrinsic lung disease, not obese with intact phrenic nerve-diaphragm axis integrity, and presence of a tracheostomy at least at the beginning of diaphragm pacing), diaphragm pacing is an optimal form of ventilatory support during wakefulness because it is portable and permits these children to participate in supervised age-appropriate activities in moderation while receiving "assisted ventilation" (23, 134, 137). In general, conservative use of diaphragm pacing is provided in active children with 12 to 15 hours per day typically recommended. The benefit of diaphragm pacing is freedom from the mechanical ventilator during daytime use. For those older patients who use the diaphragm pacers during sleep time only, the aim is to minimize the need for the mechanical ventilator and potentially remove the tracheostomy. Nonetheless, the individual who relies on diaphragm pacing will still require continuous monitoring with pulse oximetry and $P_{ET_{CO_2}}$ as well as continuous care by a highly trained registered nurse. Patients who require ventilatory support 24 hours per day should have an alternate form of ventilation for part of the day if pacers are used. Pacers can be used for daytime support of ambulatory children who require full-time ventilatory support, in combination with positive pressure ventilation at night (23, 134–137).

Obstructive apnea can be a complication of diaphragm pacing during sleep in the decannulated individual as synchronous upper airway skeletal muscle contraction does not occur with paced inspiration (143, 146). This may be overcome by adjusting settings on the pacers to lengthen inspiratory time and/or decrease the force of inspiration. Patients with CCHS using diaphragm pacing should have spare antennae in the home, as these are the components that most frequently break. It is mandatory that all individuals with CCHS who rely on diaphragm pacing have a back-up diaphragm pacer transmitter already set based on physiologic study in a center with extensive expertise in diaphragm pacing. The other advantage of the second transmitter is that for children who are paced during the day, the backup transmitter can be set to deliver optimal settings for exercise. This allows the child to use the diaphragm pacers with one transmitter during school (settings ascertained during physiologic assessment in varying levels of activity and concentration to simulate classes) and the other transmitter during a moderate level of age-appropriate activity (settings ascertained during physiologic assessment in varying levels of activity to simulate sports/activities). The mechanical ventilator would then be used for sleep time only. Some patients with CCHS may require cardiac pacemakers in addition to diaphragm pacers. Both can be used together in the same patient without in-

terference as long as the cardiac pacemaker is bipolar (134, 144, 145), thereby minimizing the potential for electromagnetic interference with the bilateral monopolar phrenic nerve electrodes.

Diaphragm pacing has the advantage that the diaphragm pacer system is small, light, battery operated, and easily portable. However, because implantation of the internal components (receivers, connecting wires, and phrenic nerve electrodes) requires a thoracic surgery and hospitalization, they should only be implanted by pediatric surgeons (ideally cardiovascular-thoracic) with extensive expertise in diaphragm pacing in children at a center with expertise in diaphragm pacing and the care of children with CCHS. If possible, the pacers should be implanted thoracoscopically. After the pacers are implanted, extensive experience in diaphragm pacer management, including the ability to set the pacers with a digital oscilloscope and surface electromyogram recordings, is required for biannual then annual comprehensive in-hospital evaluation. The goal with diaphragm pacing is to minimize the electrical stimulation while providing optimal ventilation and oxygenation.

Alcohol and Drug Abuse

With advancing technology, patients with CCHS now survive into adolescence and adult life. However, they are vulnerable to normal adolescent temptations of alcohol and drug abuse (151). For patients with CCHS, the use of drugs and alcohol can depress ventilatory control to the point where assisted ventilation is required and, in its absence, death has ensued (151). Experimentation with alcohol and drug use can produce fatal results in patients with CCHS. Therefore patients and families should be counseled about the special dangers to a patient with CCHS before the patient reaches adolescence, and continuing throughout adult life.

Pregnancy

A growing number of patients with CCHS have children of their own (46, 47). Pregnancy presents potential risks: the enlarging uterus increases respiratory load, but the mother with CCHS, already breathing at a lower minute ventilation and higher P_{CO_2} than the nonpregnant woman with CCHS (47), does not have the central respiratory drive to meet these increased ventilatory demands. Therefore, these women require frequent physiological monitoring of the adequacy of gas exchange both during spontaneous breathing while awake, and while on assisted ventilation during sleep. If a Caesarian birth is planned or occurs emergently in a pregnant woman with CCHS using diaphragm pacing without a tracheostomy, the obstetric staff should be prepared to use bilevel positive airway pressure ventilation postpartum, as diaphragm pacing is poorly tolerated after an abdominal incision (47). For offspring born to individuals with CCHS, prenatal *PHOX2B* testing allows for the anticipated infant with CCHS to be delivered in a tertiary care center with plans for immediate intubation and ventilation in the delivery room. Therefore, the authors of the ATS Statement recommend prenatal *PHOX2B* testing in any fetus who has a mother or father with CCHS, even if termination of pregnancy is not anticipated, to optimally plan for the immediate newborn care of an infant with CCHS. Plans should also be made to assure that the mother with CCHS has adequate ventilator support during labor, postpartum, and during and after any general anesthesia that may be required.

Long-term Prognosis

With modern techniques for home ventilation, most children with CCHS can have prolonged survival with a good quality of life. The mortality rate for CCHS is low in patients who are aggressively managed and followed in CCHS centers, although continuous vigilance is necessary in terms of physiologic monitoring, equipment maintenance, and battery replacement in diaphragm and cardiac pacemakers. As children with CCHS are advancing into adulthood, the development of transitional medicine programs in centers already caring for children with CCHS is essential.

A MODEL FOR TRANSITIONAL AND TRANSLATIONAL AUTONOMIC MEDICINE

When first described, CCHS was thought to be primarily a disorder of ventilatory control, insofar as patients with CCHS lacked subjective and objective responses to both hypoxia and hypercapnia. The initial challenge of compensating for a fundamental physiologic response, absent in CCHS, seemed nearly impossible to achieve in the 1970s and the earliest patients with CCHS described in the literature did not survive. However, through the years, clinicians dedicated themselves to understanding this disorder, and they achieved the ability to manage the condition. Not only did children with CCHS live with the aid of technology, they often thrived, participating in normal childhood activities including attending school, graduating from high school and college, getting married, and finding steady employment. In contrast, those receiving suboptimal and inconsistent management were left with significant disabilities and achievement of lesser accomplishments, remaining wholly dependent on their families.

As centers emerged that were dedicated to the evaluation and management of individuals with CCHS, physician-scientists were able to study cohorts of 30 or more children with CCHS to better understand the underlying nature of the disease and to more fully characterize the phenotype. Research at the handful of centers worldwide raised the awareness that children with CCHS had diffuse ANSD, not solely a control of breathing deficit, and introduced the concept that CCHS is the most severe manifestation of ANSD. In 2003, two research groups discovered that CCHS was a result of mutations in the *PHOX2B* gene. This gene, not known as causing clinical disorders, was known to basic science researchers as a gene important in the development of the ANS. We now understand that different mutations in the *PHOX2B* gene have different implications for the severity of an individual patient's disorder. Knowledge of the specific *PHOX2B* genotype or mutation guides clinicians to assess specific clinical abnormalities or risks. Some of these correlations have been described in this document, but many areas remain to be understood. Notably, CCHS stands as an example of how clinical observations suggested directions for basic science research. The resulting basic science research provided new insights into the clinical observations, and explained observations previously determined to be unknown. It is anticipated that clinical scientists and basic scientists will continue to collaborate on research to enhance knowledge in both disciplines. As children with CCHS now survive into adulthood, new insights about the long-term sequelae of ANSD will become evident. And, as more children and adults who were "missed" in earlier life are diagnosed with CCHS at an advanced age, we will likely develop a clearer understanding of the importance of aggressive conservative intervention at the youngest age possible.

Taken together, CCHS is a prototypical example of translational and transitional medicine. It represents the success of collaboration between clinicians and basic scientists and the success of the collaboration between pediatric and adult pulmo-

nologists, intensivisits, cardiologists, other specialists, and families, as children born with CCHS mature into highly functional adults because their phenotype could be anticipated and consistent management provided. In so doing, these children with a seemingly orphan disease (152), are contributing to our understanding of the basic function of the ANS as it applies to health and disease. Likewise, CCHS can serve as a cornerstone for investigating a growing number of other disorders within the rubric of ANSD (153) and, more specifically, with respiratory and autonomic disorders of infancy, childhood, and adulthood (RADICA) (41).

SUMMARY STATEMENT

1. A *PHOX2B* mutation is required to make the diagnosis of CCHS. Knowledge of the specific *PHOX2B* mutation aids in the anticipated phenotype, including severity of the ventilatory control disorder, risk of sudden cardiac death, and risk of associated disorders, such as Hirschsprung disease, neural crest tumors, as well as other adverse consequences.
2. Parents of patients with CCHS should be tested for the *PHOX2B* mutation to determine the risk of passing the *PHOX2B* mutation and CCHS on to their children (future siblings of the patient with CCHS) and to assess their own risk of ANSD, possibly requiring medical intervention.
3. Maintaining a high index of suspicion in cases of unexplained alveolar hypoventilation, delayed recovery of spontaneous breathing after sedation or anesthesia or in the event of severe respiratory infection, and seizures or neurocognitive delay will likely identify a higher incidence of the milder cases of CCHS, thereby allowing for successful intervention.
4. Patients with CCHS require lifetime mechanical assisted ventilation. They do not outgrow the disorder or the need for ventilatory support.
5. Because patients with CCHS have minimal to no lung disease, they have the widest option of ventilatory support techniques available. Although there is some controversy about the optimal modality of ventilatory support, the authors of this Statement recommend positive pressure ventilation via tracheostomy in the first several years of life when brain growth and development requiring normoxia occurs.
6. Although considerable evidence exists indicating that specific *PHOX2B* mutations dictate the severity of the disease, future studies to better understand how the *PHOX2B* genotype/mutation dictates and correlates with the CCHS phenotype are warranted.
7. Because the biology of CCHS is not completely understood, it is hoped that families of the few patients with CCHS who die will agree to autopsy, which can provide tissues to further identify and delineate biologic abnormalities. These tissues would include frozen (not fixed) brain, brainstem, carotid and aortic bodies, adrenal glands, autonomic plexus, sympathetic chain, and the entire gut.

FUTURE DIRECTIONS

Our understanding of CCHS is rapidly expanding, but it remains a work in progress. To optimally diagnose and manage patients with CCHS, advances in knowledge are required in at least the following areas. By concentrating the care of individuals with CCHS in a limited number of centers worldwide, answers to these questions will be attainable in the lifetime of current children with CCHS. Many of these studies are already underway in a US-led international cohort of patients with CCHS.

1. What is the populational incidence of *PHOX2B* mutations and contractions? Can normal gene databases be used, such as state newborn screening samples and other gene databases? What is the incidence of *PHOX2B* mutations in patients referred to adult sleep labs, adults with sleep apnea, at-risk anesthesia subjects, persons with adverse reactions to anesthesia, and complex sleep apnea patients?
2. The implications for third-generation subjects with CCHS need to be determined, especially for the 20/24 and 20/25 genotypes as well as a subset of the NPARMs. Thus, all pregnancies of patients with CCHS and mosaic parents should be monitored and reported in an attempt to determine prenatal observations (ultrasound, Doppler) and to confirm the absence of anticipation with subsequent generations.
3. What is the incidence of mosaicism in the general population and in an expanded cohort of parents of children with *PHOX2B* mutation-confirmed CCHS? Is there a CCHS phenotype of mosaic individuals? If studied with comprehensive autonomic testing will they have ANSD?
4. Is there a difference in the most common origin of gametes with *PHOX2B* mutations: paternal (sperm) or maternal (oocytes)?
5. Individuals with *PHOX2B* polyalanine contraction mutations have been described, but they are apparently asymptomatic. If studied with comprehensive autonomic testing, will they have ANSD?
6. For all future clinical studies, only subjects with *PHOX2B* mutation-confirmed CCHS should be reported (by genotype/mutation). It is clear that patients having different *PHOX2B* mutations differ substantially in terms of physiologic measures, and they cannot be assumed to be similar. In so doing, all patients with similar *PHOX2B* genotypes can be compared, and the understanding of the *PHOX2B* genotype/CCHS phenotype relationship can be advanced.
7. The authors of this Statement believe that positive pressure ventilation via tracheostomy in the first 6 to 8 years of life is associated with better oxygenation, and thus better neurologic development and function, than the use of noninvasive techniques early in life. However, this is not based on high quality evidence, so a scientific study of this hypothesis is recommended.
8. Comprehensive clinical physiologic studies of patients with CCHS that explore the consequences of ANSD should be pursued. These include such areas as airway hyperreactivity and inflammation (asthma), exercise, and sophisticated autonomic testing of all organ systems affected by the ANS.
9. Exogenous hypoxic and hypercapnic challenges have been performed on small numbers of patients with CCHS without *PHOX2B* genotyping to assess ventilatory, cardiac, and arousal responses, as well as exercise and focal brain areas on fMRI. As endogenous and exogenous hypoxemia and hypercarbia challenges are typically part of clinical management, results should be reported in large cohorts of children and adults with *PHOX2B* mutation-confirmed CCHS and analyzed by genotype.

10. Patients with CCHS have clinically apparent abnormalities in autonomic regulation of gut motility, blood pressure, cardiac rhythm, pain and anxiety perception, pupillary reactivity, temperature regulation, urinary retention, and more. The study of these abnormalities in large cohorts of children and adults with *PHOX2B* mutation-confirmed CCHS and analysis by genotype will allow for an improved understanding of the maturational issues of these systems as well as the risk for sudden cardiac death. In the process of such studies, pharmacologic intervention trials should be performed to determine if the phenotype severity can be modified.
11. Effectiveness of conservative management, including continuous overnight and hourly/periodic daytime checks of SpO_2/CO_2, continuous RN caregivers, and having two ventilators and/or two diaphragm pacer transmitters, should be assessed in large cohorts of children and adults with *PHOX2B* mutation-confirmed CCHS and analyzed by genotype.

This Statement was prepared by an *ad hoc* subcommittee of the Assembly of Pediatrics.

Members of the Subcommittee include:
Debra E. Weese-Mayer, M.D. (*Chair*)
Elizabeth M. Berry-Kravis, M.D., Ph.D.
Isabella Ceccherini, Ph.D.
Thomas G. Keens, M.D.
Darius A. Loghmanee, M.D.
Ha Trang, M.D., Ph.D.

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