Congenital Central Hypoventilation Syndrome
PHOX2B Mutations and Phenotype

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Rationale: **Congenital central hypoventilation syndrome (CCHS), a unique disorder of respiratory control associated with Hirschsprung disease (HSCR) and tumors of neural crest origin, results from polyalanine repeat expansion mutations in the paired-like homeobox (*PHOX*)*2B* gene in more than 90% of cases, and alternative *PHOX2B* mutations in remaining cases.**

Objectives: **To characterize CCHS-associated nonpolyalanine repeat mutations in *PHOX2B*, evaluate genotype–phenotype relationships, and compare clinical features of CCHS in cases with nonpolyalanine repeat mutations to those with polyalanine expansion mutations.**

Methods: **DNA from probands was analyzed by polymerase chain reaction for the common polyalanine repeat expansion. If no expansion was present, coding regions and intron–exon boundaries of *PHOX2B* were sequenced. When possible, parents and siblings were screened for the mutation found in the proband.**

Results: **Fourteen nonpolyalanine repeat mutations, including missense, nonsense, and frameshift mutations, and 170 polyalanine repeat mutations were identified in 184 CCHS probands. Both incomplete penetrance and parental mosaicism were observed within the family members of probands with nonpolyalanine repeat mutations. Increased prevalence of continuous ventilatory dependence, HSCR, and neural crest tumors was seen in the nonpolyalanine repeat group compared to those with polyalanine repeat mutations.**

Conclusions: **These data suggest that nonpolyalanine repeat mutations produce more severe disruption of *PHOX2B* function. Patients carrying these mutations should be evaluated for HSCR and neural crest tumors. Because incomplete penetrance can occur in families of CCHS probands with *PHOX2B* mutations, genetic screening of appropriate family members is indicated to evaluate reproductive risk and because asymptomatic mutation carriers may be at risk for developing alveolar hypoventilation.**

Keywords: alveolar hypoventilation; autonomic nervous system; Hirschsprung disease; polyalanine repeat

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Congenital central hypoventilation syndrome, a disease characterized by autonomic nervous system dysregulation, Hirschsprung disease, and tumors of neural crest origin, results from *PHOX2B* polyalanine repeat expansion mutations in over 90% of cases and alternatively, nonpolyalanine repeat expansion mutations in remaining cases.

What This Study Adds to the Field

This study characterizes a group of congenital central hypoventilation syndrome–associated nonpolyalanine repeat mutations in *PHOX2B* and concludes that these mutations are mostly *de novo*, although some can be inherited from a parent, predominantly affect the 3′ end of *PHOX2B*, and are generally associated with a more severe phenotype with regard to hypoventilation, Hirschsprung disease, and incidence of neuroblastoma than are the more common polyalanine repeat mutations.

Congenital central hypoventilation syndrome (CCHS; also known as the literary misnomer "Ondine's curse"; OMIM no. 209880) is a unique disorder of respiratory control that was first described in 1970 (1). Hirschsprung disease (HSCR) and/or tumors of neural crest origin (neuroblastoma, ganglioneuroblastoma, and ganglioneuroma) occur in association with CCHS in approximately 20 and 6% of cases, respectively (2). Other symptoms of diffuse autonomic nervous system dysfunction/dysregulation (ANSD) are seen frequently in CCHS and include decreased heart rate variability, an attenuated heart rate response to exercise, severe constipation, esophageal dysmotility/dysphagia, decreased perception of discomfort, pupillary abnormalities, decreased perception of anxiety, sporadic profuse sweating, and decreased basal body temperature (2).

The gene for CCHS was identified as paired-like homeobox (*PHOX*)*2B*, located on chromosome 4p12 and encoding a highly conserved transcription factor known to play a key role in the development of ANS reflex circuits in mice (3). The vast majority of individuals with CCHS are heterozygous for a polyalanine repeat expansion mutation involving the second polyalanine repeat sequence in exon 3 of *PHOX2B* (3–8) (Figure 1). Expansions are in-frame and range from 15- to 39-nucleotide insertions, resulting in expansion of the normal 20-repeat polyalanine tract to 25–33 repeats (4, 6, 8). Most expansion mutations occur *de novo* in CCHS probands. However, in the small number of families segregating CCHS, these mutations are inherited as an autosomal dominant trait (8). Also, in about 10% of cases of CCHS, an unaffected parent shows somatic mosaicism for the expansion mutation found in his/her child (8). Polyalanine repeat expansion size has been associated with severity of autonomic dysfunction (number of ANSD symptoms) (4, 8), increased R–R interval on Holter monitoring (7), and severity of ventilatory dependence (4, 8). Four in-frame polyalanine repeat deletion polymorphisms, with 7, 13, 14, and 15 repeats, have been identified in the normal population and do not appear to cause CCHS (3–5, 8, 9).

A small number (less than 10%) of subjects with CCHS do not have a polyalanine repeat expansion but do have other mutations in *PHOX2B* (3–6, 8, 10) with differing mutational mechanisms and effects on the predicted protein structure. These nonpolyalanine repeat mutations are of interest in defining

(*Received in original form February 28, 2006; accepted in final form August 24, 2006*)

Supported by the Spastic Paralysis and Allied Diseases of the Central Nervous System Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International (E.M.B.-K.), the Joseph Tyler Gertler SIDS Research Fund (D.E.W.-M.), and the Justin Carl Suth SIDS Research Fund (D.E.W.-M.).

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Am J Respir Crit Care Med Vol 174. pp 1139–1144, 2006
Originally Published in Press as DOI: 10.1164/rccm.200602-305OC on August 3, 2006
Internet address: www.atsjournals.org

functionally important parts of the PHOX2B protein and the effects of different mutational mechanisms on clinical manifestations of CCHS, particularly the severity of alveolar hypoventilation, and the presence of Hirschsprung disease and tumors of neural crest origin. The goal of this project was, thus, to identify the spectrum of nonpolyalanine repeat *PHOX2B* mutations in our series of subjects with CCHS, to determine whether these mutations tend to be inherited or *de novo*, to evaluate the predicted effects of these mutations on the PHOX2B protein, and to compare clinical features of CCHS in subjects with nonpolyalanine repeat mutations with those seen in the CCHS cohort with the common polyalanine repeat expansion mutation. An abstract describing a portion of this work was presented previously (11).

METHODS

CCHS subjects were ascertained through the CCHS clinical management and research program at Rush University Medical Center (Chicago, IL), or were identified when blood or DNA was sent to the Rush Molecular Diagnostics Laboratory from referring physicians or laboratories for DNA testing for the *PHOX2B* polyalanine repeat expansion mutation to confirm a clinical diagnosis of CCHS. Subjects in the CCHS program were referred by physicians or through self-referral of families, and clinical information and results of genetic testing provided by physicians and families was entered into a comprehensive CCHS database. For samples referred to Molecular Diagnostics, referring professionals submitted a lab intake sheet accompanying DNA samples for clinical testing. The intake sheet contained basic information regarding presence or absence of Hirschsprung disease and neural crest tumors, and whether the patient required continuous ventilatory dependence or dependence during sleep only. When a polyalanine expansion mutation was identified, the laboratory director (E.B.-K.) contacted the referring professional to relay results and verify information on the intake form. Clinical information and genotype (but no personal identifying information) was entered into a database maintained within the lab. Subjects with clinical features typical of CCHS by physician report and by review of medical records (D.E.W.-M.), but who were not shown to have a typical expansion mutation, were offered participation in the CCHS research project so that *PHOX2B* gene sequencing could be obtained. All subjects (or guardians) participating in the research project for *PHOX2B* sequencing signed an informed consent document for CCHS gene analysis and records review. Samples in this cohort were received predominantly from the United States, with less than 10% of samples coming from abroad.

The polyalanine repeat coding sequence in exon 3 of *PHOX2B* was analyzed for all subjects as described previously (8). If no expansion was seen, the entire coding region and intron–exon boundaries of *PHOX2B* were sequenced after polymerase chain reaction amplification of each exon followed by column purification of polymerase chain reaction products and automated cycle sequencing (8).

RESULTS

A total of 184 samples from individuals with the characteristic phenotype of CCHS were analyzed. A subgroup of 67 of these cases was presented previously (8). Of that group, 65 were reported to have a *PHOX2B* common polyalanine repeat mutation, 1 was found to have a nonpolyalanine repeat mutation, and 1 presented a normal coding for 20 polyalanine repeats in both alleles of the *PHOX2B* gene. We subsequently identified a labeling error at the laboratory of origin for the sample from the subject with the normal result. A new sample from the same subject demonstrated that the subject was heterozygous for a *PHOX2B* polyalanine repeat expansion mutation with 30 repeats. In the current expanded cohort, of 184 total samples analyzed at Rush University Medical Center with typical features of CCHS, 170 (92%) were heterozygous for the polyalanine repeat expansion mutation in *PHOX2B*, with the size of the repeat tract ranging from 25 to 33 repeats. A deletion polymorphism of 15 repeats was also found in one individual (1%) heterozygous for a repeat expansion mutation, similar to the frequency of this variant in the normal population. Fourteen probands with typical CCHS but not possessing a repeat expansion mutation were heterozygous for other mutations in *PHOX2B*. Missense mutations occurred in four probands (two mutations), one proband had a nonsense mutation, and nine probands (four mutations) had frameshift mutations, all located at the end of exon 2 or within exon 3 (Figure 1). Mutations and the resultant predicted effect on the PHOX2B protein are shown in Table 1. Results of *PHOX2B* analyses performed on the accessible parents and siblings of these 14 probands, if available, are also shown in Table 1. Six of nine mutations for which both parental samples were available and negative were apparently *de novo*, but the missense mutation in proband 2 was also present in the reportedly asymptomatic mother and one frameshift mutation (proband 6) was present in the reportedly asymptomatic mother and a sister with Hirschsprung disease but not CCHS, suggesting variable penetrance for this particular *PHOX2B* mutation. Further, the mother of proband 9 showed low-level somatic mosaicism for the 35-bp deletion. Although only the mother's sample was available for proband 1, the mother was a carrier of the missense mutation (same mutation as for proband 2).

Other nonpolyalanine repeat expansion mutations with significant effect on the PHOX2B protein have been reported as associated with CCHS by groups in Italy (4, 10), Japan (5), and France (6). A summary of all mutations identified by other groups is shown in Table 2. Data from subjects in this report (Table 1), and those in Table 2, reveal that 32 individuals with CCHS and nonpolyalanine repeat mutations in *PHOX2B* have been described worldwide, and mutations include mostly frameshift mutations (24 of 32, 75%), but also a nonsense mutation (1 of 32, 3%), missense mutations (6 of 32, 19%), and a missense mutation with stop codon alteration (1 of 32, 3%). The 38- and 35-bp deletions begin in the first codon of the polyalanine repeat, and these are recurring mutations (found in seven and three probands in unrelated families, respectively) identified by investigators in different countries. The G422A and A428G mutations have also each been found in several unrelated probands, and these are the only two missense mutations yet identified in CCHS probands. All CCHS-associated mutations at our center and elsewhere are found at the end of exon 2 or in exon 3 (Figure 1). Interestingly, the Italian 618delC mutation, located in the same area of *PHOX2B* as the single base-pair deletion in our 577delG family, was inherited from the asymptomatic mother (4), consistent with our finding that some frameshift mutations can be variably penetrant. It should be noted, however, that comprehensive physiologic testing of these family members has not been reported.

Review of available clinical information from our cohort and from subjects reported worldwide (4-6, 10) (Figure 2) with *PHOX2B* mutations revealed that HSCR was reported in 87% of cases (26/30) with a non-polyalanine repeat mutation, and 17% of cases (60/345) with a polyalanine repeat mutation. Continuous ventilatory dependence was reported in 79% of cases (15/19) with a non-polyalanine repeat mutation and 34% of cases (66/193) with a polyalanine repeat mutation. Neural crest tumors were reported in 50% of cases (8/16) known to be over one year of age with a non-polyalanine repeat mutation, and 1% of cases (4/279) with a polyalanine repeat mutation. All polyalanine repeat probands with tumors were heterozygous for large (either 31 or 33 repeat) expansion mutations. Thus, a much higher rate of HSCR, higher frequency of continuous ventilatory dependence, and more frequent neural crest tumors is identified in the nonpolyalanine repeat mutation group compared to those with a polyalanine repeat expansion, both in our series of patients and

TABLE 1. NONPOLYALANINE REPEAT EXPANSION MUTATIONS IN *PHOX2B* AMONG PROBANDS WITH CCHS*

Proband	Mutation Location	Mutation in Genomic Sequence	cDNA Change	Mutation Type	Protein Change	Mutated Protein Size
Proband 1	Exon 2	G2142A	G422A	Missense CGA-CAA,	arg-gln	314
Mother	Exon 2	G2142A	G422A			
Proband 2	Exon 2	G2142A	G422A			
Mother	Exon 2	G2142A	G422A			
Father	Normal					
Proband 3	Exon 2	A2148G	A428G	Missense CAG-CGG	gln-arg	314
Mother	Normal					
Father	Normal					
Proband 4	Exon 2	A2148G	A428G			
Mother	Normal					
Father	Normal					
Proband 5	Exon 3	A3209G	A463T	Nonsense AAG-TAG	Terminates at K155	154 (truncated)
Proband 6	Exon 3	3323delG	577delG	Frameshift	Abnormal sequence from site of deletion, polyalanine repeat obliterated	307 (slightly truncated)
Mother	Exon 3	3323delG	577delG			
Sister	Exon 3	3323delG	577delG			
Father	Normal					
Proband 7	Exon 3	3363insT	617insT	Frameshift	Abnormal sequence from site of insertion, polyalanine repeat obliterated	358 (elongated)
Mother	Normal					
Father	Normal					
Proband 8	Exon 3	3468del35	722del35	Frameshift	Abnormal sequence from site of deletion, polyalanine repeat obliterated	346 (elongated)
Proband 9	Exon 3	3468del35	722del35			
Mother	Exon 3 mosaic†	3468del35	722del35			
Father	Normal					
Proband 10	Exon 3	3468del38	722del38	Frameshift	Abnormal sequence from site of deletion, polyalanine repeat obliterated	345 (elongated)
Mother	Normal					
Father	Normal					
Proband 11	Exon 3	3468del38	722del38			
Mother	Normal					
Father	Normal					
Proband 12	Exon 3	3468del38	722del38			
Proband 13	Exon 3	3468del38	722del38			
Proband 14	Exon 3	3468del38	722del38			
Mother	Normal					
Father	Normal					

* All Probands except for Proband 4 have Hirschsprung disease.
† The mother of Proband 9 showed a low level (10%) somatic mosaicism for the 722del35 mutation found in her child.

when published data from all other groups globally were combined with our data (4–6, 10) (Figure 2).

DISCUSSION

This article describes *PHOX2B* mutation analysis in a large cohort of 184 subjects referred to Rush University Medical Center with the classical CCHS phenotype. Ninety-two percent of subjects with CCHS are heterozygous for the polyalanine repeat expansion mutation in exon 3. However, in this series, all 14 subjects presenting with typical features of CCHS who did not have a polyalanine repeat expansion mutation in *PHOX2B* had a different mutation in the *PHOX2B* gene. The 100% mutation detection rate in this cohort most likely has to do with the careful

TABLE 2. NONPOLYALANINE REPEAT EXPANSION MUTATIONS IN *PHOX2B* ASSOCIATED WITH CONGENITAL CENTRAL HYPOVENTILATION SYNDROME FROM THE LITERATURE

Study (Ref.)	Number of Probands with Mutation	Mutation Location	cDNA Change	Mutation Type	Protein Change	Mutated Protein Size (*kD*)
France (6)	1	Exon 2	G422A	Missense	Arg→Gln	314
France (6)	1	Exon 2	A428G	Missense	Gln→Arg	314
France (6)	1	Exon 3	606insA	Frameshift	*	358
France (6)	1	Exon 3	618insC	Frameshift	*	358
Italy (4)	1	Exon 3	618delC	Frameshift	*	307
France (6)	1	Exon 3	692delG	Frameshift	*	307
France (6)	1	Exon 3	693del8	Frameshift	*	355
France (6)	1	Exon 3	689dup8	Frameshift	*	310
France (6)	1	Exon 3	722del35	Frameshift	*	346
France (6); Italy (4)	3	Exon 3	722del38	Frameshift	*	345
Japan (5), Italy (4)	2	Exon 3	866insG	Frameshift	†	358
France (6)	1	Exon 3	931del5	Frameshift	†	356
Italy (10)	1	Exon 3	930insG	Frameshift	†	358
France (6)	1	Exon 3	936insT	Frameshift	†	358
France (6)	1	Exon 3	A945C	Stop codon	Obliterates stop codon	355

* Abnormal sequence from site of deletion or insertion, polyalanine repeat obliterated.
† Abnormal sequence from site of deletion or insertion.

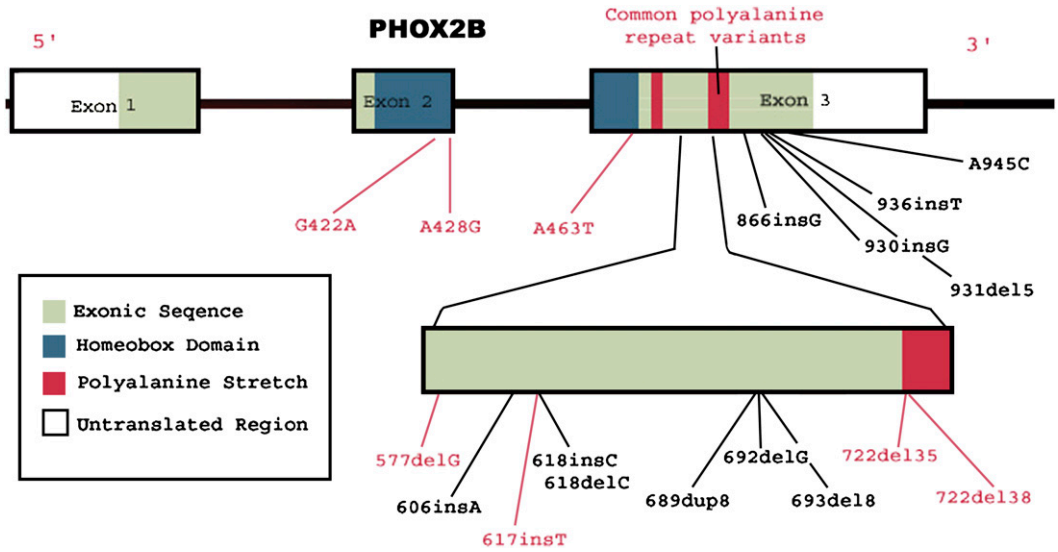

Figure 1. Location of all congenital central hypoventilation syndrome (CCHS)–associated mutations described to date in *PHOX2B*. Note all thus far identified mutations are found at the very 3′ end of exon 2 or in exon 3. Mutations noted in *red* were identified in this series. Additional mutations identified elsewhere are also noted. All polyalanine repeat mutations are located within the second polyalanine stretch of exon 3.

clinical review and restriction of inclusion to subjects who met strict criteria for CCHS (2). It is possible that occasional additional subjects with nonpolyalanine repeat mutations in *PHOX2B* would be identified if nontypical subjects with CCHS-spectrum symptoms were analyzed.

Mutations described here and worldwide include missense, nonsense, and frameshift mutations, but all are located at the 3′ end of the *PHOX2B* gene from the last 6 bp of exon 2 to the end of exon 3. Both of the missense mutations in our series, A428G and G422A, occurred in two unrelated CCHS probands. These same mutations were identified in the French series (6), suggesting these mutations have arisen several times on independent backgrounds. Furthermore, it is worth noting that these two mutations are the only described missense mutations identified in *PHOX2B* and occurring in cases of CCHS. Both mutations alter a highly conserved amino acid in the PHOX2B sequence; however, they also both alter residues predicted to be important in the splice donor consensus sequence for splicing of exons 2 and 3

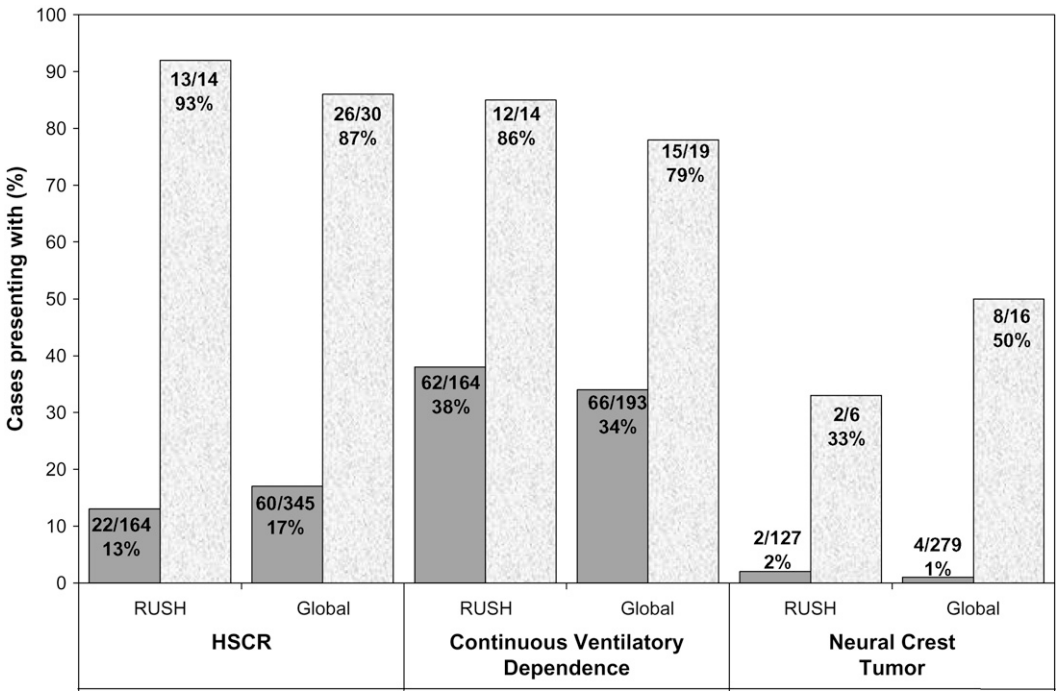

Figure 2. Rate of Hirschsprung disease (HSCR), continuous ventilatory dependence, and neural crest tumors in CCHS probands with and without polyalanine repeat expansion mutations in *PHOX2B*. Clinical information was available from 178 Rush University Medical Center cases for whom phenotypic information was available (164 polyalanine repeat mutations and 14 nonpolyalanine repeat mutations). *HSCR*: HSCR was identified/reported in 26 of 30 cases with a nonpolyalanine repeat mutation (1, Japan; 3, Italy; 14, Rush; 12, France), and 60 of 345 cases with a polyalanine repeat mutation (7, Japan; 22, Italy; 164, Rush; 152, France). Ventilatory dependence awake and asleep: continuous ventilatory dependence was identified/reported in 15 of 19 cases with a nonpolyalanine repeat mutation (1, France; 1, Japan; 3, Italy; 14, Rush), and 66 of 193 cases with a polyalanine repeat mutation (7, Japan; 22, Italy; 164, Rush). Neural crest tumor data were derived from cases for which information was available and in which the proband had survived at least the first year of life. *Neural crest tumor*: Neural crest tumors were identified/reported in 8 of 16 cases known to be over one year of age with a nonpolyalanine repeat mutation (6, Rush; 10, France), and in 4 of 279 cases with a polyalanine repeat mutation (127, Rush; 152, France). *Note*: In the latter cases, all polyalanine repeat probands with tumors had large (either 31 or 33 repeat) expansion mutations. There is a possibility for minor overlap in subjects among the polyalanine repeat cases among these reports as previously noted (12). However, overlap among the nonpolyalanine expansion cases is unlikely. *Dark shaded bars*, polyalanine repeat mutations; *light shaded bars*, nonpolyalanine repeat mutations.

and may actually exert their effect through a splicing defect. Altered splicing would produce an abnormal PHOX2B C terminus, similar to the abnormal proteins observed with the frameshift mutations. Further work is needed to analyze RNA splicing with these mutations in a tissue expressing PHOX2B. There is another multiply recurrent mutation in our series, also reported in both French (6) and Italian (4) cohorts, that involves deletion of 35 or 38 bp from the beginning of the polyalanine repeat stretch, which may be generated through a specific mutational mechanism during replication of the polyalanine repeat.

Most nonpolyalanine repeat mutations arise *de novo*, although they can be inherited and variably penetrant in families. Family members who have HSCR but not CCHS may carry these mutations, and some carriers may be asymptomatic as seen in families of probands 1, 2, and 6. Also, low-level somatic mosaicism for a large deletion was observed in the mother of proband 9. Taken together, this information suggests that recurrence risk can exist in families in which the CCHS proband has a nonpolyalanine repeat mutation, and emphasizes the importance of screening parents once a mutation has been identified in their child. Furthermore, apparently asymptomatic parents or siblings of a CCHS child who carry the child's mutation in *PHOX2B* may be at risk for developing hypoventilation in sleep or a later, adult-onset atypical CCHS phenotype (13). Identification of mutation carriers in these families allows for appropriate clinical monitoring and early implementation of treatment, if required.

As was also observed in the CCHS cohort from France (6), nonpolyalanine repeat mutations appear virtually always to result in Hirschsprung disease and to have a high rate of neural crest tumors in patients who live past one year of age, suggesting that many of these mutations are more disruptive to the function of *PHOX2B* than the polyalanine repeat expansions. Individuals with CCHS and a nonpolyalanine repeat mutation, thus need to be monitored closely for emergence of a neural crest tumor, specifically neuroblastoma. Nonpolyalanine repeat mutations appear to be divided into two groups, according to clinical presentation. The majority of these mutations appear to produce very severe disease with continuous full ventilatory support and HSCR with extensive gut involvement, and in those over one year of age an increased tumor risk (11 patients in our series including all large deletions and one other +1 frameshift, one nonsense mutation and one missense mutation). There is a minority however which also has a very high incidence of HSCR but milder disease, and incomplete penetrance in the families of three patients with a particular missense mutation or frameshift. Thus, presence of HSCR and a CCHS phenotype is a strong predictor of a nonpolyalanine repeat mutation in *PHOX2B* when polyalanine repeat expansion screening is negative.

Lack of identification of mutations in exon 1 and most of exon 2 (Figure 1) may suggest one of two possibilities, given that disruption of the 5′ end of the gene is more likely to lead to *PHOX2B* haploinsufficiency caused by lack of production of any PHOX2B protein due to nonsense-mediated decay of mRNA. One possible interpretation would be that haploinsufficiency may produce a more severe reduction of activity than that which accompanies a marginally functional 3′-mutated protein. Consequently, haploinsufficiency induced by mutations at the 5′ end of the gene would be incompatible with life. Alternatively, and more likely, haploinsufficiency is insufficient to cause the phenotype and therefore individuals with 5′ mutations in *PHOX2B* do not have CCHS and are not ascertained. In this scenario, the mutations in distal exon 2 and exon 3 of *PHOX2B* result in a dominant negative or gain-of-function effect. Dominant negative effects have been proposed as a mutational mechanism for other transcription factors in which polyalanine expansion mutations cause disease, such as *HOXD13* mutations in synpolydactyly syndrome (14). These effects may occur because of the mutant PHOX2B protein directly inhibiting the function of the normal protein during the normal dimerization process required for the transcriptional regulation function of *PHOX2B*, or as a result of sequestering of the normal protein in nuclear or cytoplasmic aggregates, as has been demonstrated for other autosomal transcription factors (*HOXD13*, *FOXL2*, and *ZIC-2*) with associated polyalanine repeat expansions (14–17).

Support for the dominant negative/gain-of-function hypotheses over the haploinsufficiency model for *PHOX2B* mutations in CCHS comes from prior reports of two individuals with interstitial deletions of 4p12 containing the *PHOX2B* region (18, 19) who therefore have haploinsufficiency for *PHOX2B* but do not have CCHS. Also, mice heterozygous for a targeted *phox2b* deletion show only a mild respiratory phenotype, limited to the newborn period (20). Humans with a similar phenotype would be unlikely to be ascertained clinically, although they might have an increased risk of sudden infant death syndrome or sudden unexplained death in childhood. Further support for the dominant negative hypothesis comes from the observation of sequestering of the normal *PHOX2B* protein in nuclear aggregates from cells coexpressing normal and mutant *PHOX2B* (10). However, one study found that a 10-fold excess of the mutant protein was required before significant amounts of the normal protein were observed in aggregates (21). Thus, the presence of the aggregates may indicate a gain-of-function mechanism producing cellular toxicity or dysfunction.

Both polyalanine repeat mutations and nonpolyalanine repeat mutations in *PHOX2B* impair transcriptional function, and transcriptional impairment increases with mutation size for polyalanine repeat mutations (10, 21). For nonpolyalanine repeat mutations, the least impairment of transcriptional function, comparable to activity from the smallest (five-repeat) polyalanine expansion, was seen with the 618delC mutation (10), which results in a mutated protein similar to our 577delG mutant, both –1 frameshifts occurring in the same area of the protein. This is consistent with the finding that the 618delC (4), 577delG (this article), and five-repeat polyalanine expansion mutations can all be associated with incomplete penetrance in families of the CCHS proband or adult-onset disease (13). Thus, specific types and locations of mutations may be more prone to present with variable penetrance, based on a milder effect on PHOX2B-mediated transcription. The G422A mutation also shows incomplete penetrance, but the effect on PHOX2B function remains to be tested.

Polyalanine expansions resulted in cytoplasmic sequestration of the mutant protein, whereas this effect was not seen with frameshifts (10, 21). Frameshift mutations in fact resulted in enhanced *PHOX2A* transcription, whereas polyalanine repeat mutations reduced *PHOX2A* transcription (10). The different pattern of transcriptional dysregulation and mutant protein aggregation observed with frameshifts suggests that these mutations exert effects on *PHOX2B* target promoters through a somewhat different mechanism than the more prevalent polyalanine repeat mutations. Excessive activation of *PHOX2A* was proposed to be a potential factor in the high incidence of neural crest tumors in CCHS subjects with nonpolyalanine repeat mutations. In any case, given the more severe hypoventilation observed in most subjects with nonpolyalanine repeat mutations, and near universal presence of HSCR, many of these mutations seem to result in cellular disease mechanisms distinct from or in addition to those operant in the presence of a polyalanine repeat mutation, thus producing a larger and more generalized impact on *PHOX2B* function.

Continued identification of nonpolyalanine repeat mutations in *PHOX2B* in individuals with symptoms in the CCHS/ANSD spectrum will yield further information about the importance of different domains in the PHOX2B protein and will help elucidate the genetic mechanisms through which mutations in *PHOX2B* result in disease.

***Note added in proof*:** Subsequent to the acceptance of this work, 17 more CCHS subjects were identified for a total of 201 *PHOX2B* mutation-confirmed cases of CCHS at Rush University Medical Center. These new cases included 15 with polyalanine repeat mutations and 2 with nonpolyalanine repeat mutations (722del38 and 689dup8).

***Conflict of Interest Statement*:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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