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Congenital Central Hypoventilation Syndrome: *PHOX2B* Genotype Determines Risk for Sudden Death

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Summary. Objective: Children with Congenital Central Hypoventilation Syndrome (CCHS) have cardiovascular symptoms consistent with the autonomic nervous system dysregulation/dysfunction (ANSD) phenotype. We hypothesized that children with CCHS would have a relationship between *PHOX2B* genotype and two clinically applicable cardiovascular measures of ANSD: duration of longest r-r interval and longest corrected QT interval (QTc). Materials and Methods: We studied 501 days of Holter recordings from 39 individuals with *PHOX2B* mutation-confirmed CCHS, and analyzed longest r-r and QTc intervals with respect to *PHOX2B* genotype. Results: We determined that longest r-r interval varied by genotype ($P = 0.001$), with a positive correlation between repeat number and longest r-r interval duration ($P = 0.0007$). Number of children with a longest r-r interval value $\geq$3 sec varied by genotype ($P < 0.0001$): 0% with the 20/25 genotype, 19% with the 20/26 genotype, and 83% with the 20/27 genotype. Though longest QTc interval did not vary by genotype ($P = 0.09$), all children with CCHS had at least one Holter with a QTc interval >450 msec, and percent of time with QTc >450 msec exceeded published values. The proportion of subjects who received a cardiac pacemaker due to prolonged r-r interval was greater for the children with the 20/27 genotype (67%) than the 20/25 (0%) or 20/26 genotype (25%) ($P = 0.01$). Among three children who did not receive a cardiac pacemaker, but who had r-r intervals $\geq$3 sec, two died suddenly. Conclusions: These results confirm a disturbance of cardiac autonomic regulation in CCHS, indicate that *PHOX2B* genotype is related to the severity of dysregulation, predict the need for cardiac pacemaker, and offer the clinician the potential to avert sudden death. **Pediatr Pulmonol. 2008; 43:77–86.**

Key words: *PHOX2B* gene; transient asystoles; QTc.

INTRODUCTION

Congenital Central Hypoventilation Syndrome (CCHS) is one of a growing number of disorders characterized by autonomic nervous system dysfunction/dysregulation (ANSD).[1] Individuals with CCHS typically present in the newborn period with alveolar hypoventilation during sleep (though more severely affected cases hypoventilate in sleep and wakefulness), an attenuated or absent response to hypoxemia and hypercarbia, evidence of diffuse autonomic dysfunction/dysregulation,[2] and a characteristic facies.[3] More recently, presentation of CCHS in adulthood has also been identified.[4,5] Paired-like homeobox (*PHOX*)*2B* is the disease-defining gene for CCHS.[6–9] Individuals with the CCHS phenotype are heterozygous for a polyalanine (PA) repeat expansion mutation in the *PHOX2B* gene in 92% of cases and non-PA repeat mutations in *PHOX2B* in 8% of cases.[6–11] The 15–39 nucleotide insertions result in expansion of the normal 20 repeat polyalanine tract to 25–33 repeats and genotypes of 20/25 to 20/33. The non-PA repeat mutations can include missense, nonsense, and frameshift mutations.

Patients with CCHS display several associated cardiovascular symptoms, reflecting ANSD. These include disturbances of heart rate and rhythm as well as blood pressure. Although baseline heart rate in patients with CCHS does not differ from controls,[12–17] the relative increase above the mean baseline heart rate is attenuated

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with exercise.[15] Furthermore, heart rate variability is decreased.[12,16] Children with CCHS exhibit an increased frequency of arrhythmia, primarily sinus bradycardia and transient asystole, with documented pauses as long as 6.5 sec in CCHS versus 1.4 sec in controls.[16] Children with CCHS exhibit lower blood pressure values during wakefulness and higher blood pressure values during sleep (vs. controls), indicating attenuation of the normal sleep-related blood pressure decrement.[14] They also demonstrate a limited capacity to elevate their blood pressure on standing and head-up tilt positions, and do not demonstrate the normal standing-related blood pressure overshoot.[17] Taken together, these data indicate that the cardiovascular system in individuals with CCHS manifests limitations in the normal flexibility of the system to respond to diverse stimuli. In contrast, healthy children without CCHS rarely demonstrate prolonged asystole and sinus pauses. In a subsequent analysis of data from the Collaborative Home Infant Monitoring Evaluation (CHIME) study of 1,079 infants, 1.9% of cardiorespiratory events had an r-r interval $\geq$1 sec, accounting for $<$2% of the infants.[18] In a study of 134 healthy full-term infants in the first 10 days of life, 2.2% show pauses $\geq$1 sec, with the longest being 1.43 sec.[19] Among 104 English school children, pauses longer than 1.82 sec are suggested to indicate abnormal sinus node function.[20] One teenager among 360 Japanese children is identified with a sinus pause longer than 3 sec.[21] However, in 35 adolescent and young adult male endurance athletes, sinus pauses of greater than 2 sec are documented in 37.1% of the subjects (vs. 5.7% of nonathlete controls).[22] Taken in perspective, large studies of healthy normal children and young adults rarely demonstrate sinus pauses or transient asystoles, with the exception of endurance athletes. However, small studies of children with CCHS (sample size 7–14 subjects), studied in an era before *PHOX2B* genetic testing, often had transient asystoles on Holter recording.

We and others have previously demonstrated a relationship between *PHOX2B* genotype and the phenotype of ANS dysfunction in CCHS, such that children with larger polyalanine repeat expansion mutations in *PHOX2B* display more diffuse dysfunction of the ANS and more severe respiratory compromise than children with smaller mutations.[7,9] Given that children with CCHS demonstrate cardiovascular symptoms consistent with ANSD, we conducted this study to evaluate potential relationships between the *PHOX2B* genotype in children with CCHS and two clinically applicable cardiovascular measures of autonomic nervous system dysfunction/dysregulation: duration of the longest r-r interval and the longest corrected QT interval (QTc). We selected r-r interval because it is the basis on which clinical decisions are made regarding cardiac pacemaker implantation. The QTc was selected as an accessible measure of autonomic dysregulation. The long term goal is identification of those individuals at greatest risk for asystole-induced decreases in cerebral perfusion and resultant extreme outcomes such as sudden death or severe neurocognitive impairment.

MATERIALS AND METHODS

Study Subjects

All children were identified through the Rush Children's Hospital CCHS Center. Children with the CCHS phenotype who were followed longitudinally with serial Holter recordings and who were known to have *PHOX2B* mutation-confirmed CCHS were identified. Ethnicity was assigned based on self-report. IRB approval was obtained for the assessment of this cohort. Because the studies were obtained as part of standard care, individual consent was not required by the IRB.

Pertinent Clinical Information

The dates of cardiac and diaphragm pacemaker implantation were obtained from the medical records, as was the recommendation for daily duration of support with artificial ventilation. Recommendation of 24 hr ventilatory support was selected rather than use of 24 hr support in order to accurately reflect the severity of the disease phenotype, rather than reflecting the level of patient compliance. Indication of postnatal hypoxemia as evidenced by pulmonary hypertension on echocardiogram and/or clinical seizures was also reported.

PHOX2B Results

DNA preparation and *PHOX2B* genotype analysis were performed as previously described.[7] Genetic results for the patients presented here have been previously reported.[7,10]

Holter Recording and Analysis

Continuous ambulatory electrocardiograms (ECG) were obtained in each subject using five silver/silver chloride surface electrodes. The Holter recordings were all performed under the same conditions of ventilatory support for a given subject. Five electrodes were placed for each Holter recording with locations on the sternum, the left mid-axillary line, the right mid-axillary line (all at the level of the 5th intercostal space), at the level of the manubrium, and at the level of the left first costal cartilage. The electrodes recorded 3 channels and produced derived 12-lead ECG data. From years 1990 to 2005, a Zymed analog recorder used a field-proven capstan drive mechanism with a tape speed of 1 mm/sec (Zymed, Camarillo, CA), and from years 2005 to 2006, the Philips DigiTrak Plus used a digital recorder (Philips Medical Systems, Bothell, Washington), both capable of

continuous recording for 24 hr. The in-hospital clinical care protocol for children with CCHS includes Holter monitoring for three consecutive 24 hr periods, yielding 72 hr continuous Holter recordings during each hospital admission at Rush University Medical Center.

To analyze the Holter recordings, the defining points of a wave (including the QRS complex, the beginning of the P wave, and the end of the T wave) were determined. The sampling rate for signal acquisition was 175 samples/sec. Heart rate minimum, average, and maximum data were calculated in 7 sec intervals. The longest r-r interval was calculated by the software, and confirmed by visual inspection without knowledge of subject diagnosis by an experienced Pediatric Cardiologist (B.A.S.). The QTc and percentage of QTc intervals greater than 450 msec were added to the analysis software in the last 21 months of the Holter study.

The following variables were collected from each Holter report: date, time, and patient age at recording, minimum, mean, and maximum heart rates, maximum r-r interval, QTc minimum, mean, and maximum, and the percentage of Holter duration with QTc intervals greater than 450 msec. Data collected after cardiac pacemaker implantation were not included in the analysis except for the occurrences when the cardiac pacemaker battery was clearly not functioning and the child experienced prolonged pauses.

Statistical Analysis

Values are summarized as means, standard deviations (SD), medians, minima and maxima or as frequency (percentage) as appropriate. In view of the variability of the extreme Holter measures (minimum heart rate, longest r-r and QTc intervals), we were not able to employ longitudinal models of monitoring measures as a function of age. Instead, we summarized by deriving the longest r-r interval and the longest QTc interval for each subject, with the age at these occurrences. We also analyzed the data collected for the first Holter recording for each patient. The purpose of analyzing and reporting data from the first Holter session is to provide a baseline for comparison to subsequent data. The differences between the first Holter session data as compared to the aggregate data emphasize the sporadic nature of the longest r-r interval and the need for longitudinal study of these patients to identify the events that heighten their vulnerability. The use of extreme values such as longest r-r could raise statistical difficulties; therefore, when we summarize experience across children we report medians and quartiles and we employ non-parametric tests. Moreover, even one r-r interval of the length seen here may be of clinical importance. The use of extreme values is of clinical relevance because decisions to implant pacemakers can be triggered by isolated extremes of the magnitude reported here. If the r-r interval is 3 sec or longer, the Pediatric Cardiologist will typically recommend cardiac pacemaker implantation. This decision would not be based on mean or median r-r values. The pauses occur suddenly and unpredictably, and so do not appear to be well reflected in composite values. Similarly, we considered the longest value for QTc to best reflect the extreme of autonomic dysfunction. The 20/25, 20/26, and 20/27 genotypes were compared using Kruskal–Wallis tests (the two cases with the non-PA repeat mutation were excluded from the genotype analysis), with the exception of the longest r-r interval categories for which Fisher's Exact test was used. *Post hoc* Wilcoxon rank sum tests are given. The possible importance of age at examination and of the number of Holter sessions was considered via graphs and Spearman correlations (data not shown), but the findings did not impact results. The potential association of number of repeats with each of five potential confounding variables (recommendation of 24 hr/day ventilation, recommendation of cardiac pacemaker, presence of diaphragm pacemaker, pulmonary hypertension, and seizures) was examined via univariate logistic regressions of each variable on the number of repeats. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Study Subjects

A total of 39 children met study entry criteria (20 females). Age at first Holter monitoring session ranged from 0.83 to 19.7 years (median 3.8 years), with a mean age of 5.5 (SD 4.9) years. Study subjects included 35 Caucasian, 2 African American, and 2 Hispanic individuals with CCHS. None of the study subjects was related to each other.

Pertinent Clinical Information

Twenty-four hour per day support with artificial ventilation was recommended for 27 of the 39 children (69%, all tracheotomized), with 19 of these children receiving diaphragm pacemakers, 5 children relying on the mechanical ventilator continuously, and 3 children with very mild hypoventilation breathing spontaneously while awake. Asleep only support was recommended for 12 of the 39 children (31%), with 2 of these children relying on diaphragm pacemakers, 8 children relying on the mechanical ventilator via a tracheostomy, and 2 children relying on nasal mask Bi-PAP. Thirteen of the 39 children (33%) were implanted with cardiac pacemakers (2 of these children had prolonged r-r intervals documented in this study but pacemaker implantation was delayed; 2 additional children experienced prolonged asystoles elsewhere after this study was terminated and received a cardiac pacemaker). Eight children (21%) had both diaphragm and cardiac pacemakers. Age range at

cardiac pacemaker implantation was 0.8–19.2 years (median 11.6 years), with a mean age of 10.3 (SD 6.9) years. Holter data collected after cardiac pacemaker implantation and verified pacemaker function were excluded from the analysis. Two children demonstrated their longest r-r intervals during surveillance Holters, indicating cardiac pacemaker battery depletion. Fifteen children (38%) had echocardiogram evidence for pulmonary hypertension, and 21 (54%) had a history of clinically apparent seizures. Thirty seven of the 39 individuals with CCHS in this study are alive at the time of this report. One death occurred during nocturnal sleep on a mechanical ventilator and one death occurred during wakefulness with diaphragm pacing in previously stable young adults (genotypes 20/27 and 20/26, respectively).

PHOX2B Results

PHOX2B genetic testing (Molecular Diagnostics Laboratory, Rush University Medical Center) revealed the following distribution by genotype: 20/25 (n = 6 subjects), 20/26 (n = 16 subjects), 20/27 (n = 12 subjects), 20/30 (n = 2 subjects), 20/33 (n = 1 subject), and a non-PA repeat *PHOX2B* A428G mutation (n = 2 unrelated subjects).

Holter Recording and Analysis

A total of 501 Holter recordings were collected from the 39 children as part of routine patient care between 1990 and 2006. Of these, 45 Holter recordings from 16 children included QTc measures. Due to small sample sizes, the 2 children with non-PA repeat mutations were excluded from the analysis, but the data are provided in the tables.

Heart Rate Analysis

First Holter Recording

Baseline values for the mean, minimum, and maximum heart rate for the first Holter recordings of each child are provided in Table 1. Age at first Holter recording did not vary by genotype ($\chi^2_{2df} = 1.422$, $P = 0.5$). Minimum heart rate varied by genotype ($\chi^2_{2df} = 6.89$, $P = 0.03$), with lowest values obtained for the subjects with the 20/27 genotype (genotype 20/25 vs. 20/27 comparison $P = 0.02$, 20/25 vs. 20/26: $P = 0.07$, 20/26 vs. 20/27: $P = 0.2$). Mean heart rate did not vary by genotype ($\chi^2_{2df} = 1.82$, $P = 0.4$). Maximum heart rate did not vary by genotype ($\chi^2_{2df} = 4.31$, $P = 0.1$).

Holter Recording With Longest r-r Interval

Baseline values for the mean, minimum, and maximum heart rate for the Holter recordings during which the longest r-r interval was recorded for each child are provided in Table 2. Age did not vary by genotype ($\chi^2_{2df} = 5.14$, $P = 0.08$). Minimum heart rate varied by genotype ($\chi^2_{2df} = 11.70$, $P = 0.003$); subjects with the 20/27 genotype had lower minimum heart rates than the subjects with the 20/25 or the 20/26 genotypes. *Post hoc* comparisons support a difference between 20/25 and 20/27 ($P = 0.002$) and between 20/25 and 20/26 ($P = 0.02$), but not 20/26 versus 20/27 ($P = 0.07$). Mean heart rate did not vary by genotype ($\chi^2_{2df} = 2.22$, $P = 0.3$). Maximum heart rate did not vary by genotype ($\chi^2_{2df} = 2.48$, $P = 0.3$).

R-R Interval Data

Data for the first Holter recording and the recording with the longest r-r interval are provided in Tables 1 and 2, respectively. Figure 1 indicates the longest r-r interval by age relative to *PHOX2B* genotype. Due to small sample sizes, the five children with polyalanine repeat expansions longer than 20/27 or with a nonpolyalanine repeat mutation do not appear in Table 1 or Table 2 in detail, but are discussed below.

First Holter Recording

Values for the longest r-r interval on the first Holter recording by *PHOX2B* genotype are provided in Table 1. The longest r-r interval on the first Holter did not vary by genotype ($\chi^2_{2df} = 4.99$, $P = 0.08$).

TABLE 1— First Holter Recording by *PHOX2B* Genotype From 39 Subjects With CCHS

Characteristic	20/25	20/26	20/27	All 39 cases
Number of subjects	6	16	12	39
Age at baseline	3.1 ± 2.2 (1.4, 2.04, 6.4)	5.5 ± 6.2 (0.08, 1.9, 19.7)	6.1 ± 4.2 (0.7, 6.8, 11.7)	5.5 ± 4.9 (0.08, 3.8, 19.7)
Minimum heart rate (bpm)	64.3 ± 12.4 (47, 64, 85)	53.7 ± 10.4 (34, 56, 73)	49.0 ± 9.0 (37, 46, 66)	55.1 ± 13.7 (34, 55, 94)
Mean heart rate (bpm)	107.2 ± 16.6 (77, 111, 125)	114.7 ± 21.3 (83, 112, 158)	103.8 ± 12.2 (86, 102, 121)	109.4 ± 17.6 (77, 108, 158)
Maximum heart rate (bpm)	171 ± 20 (138, 178, 194)	174 ± 26 (136, 176, 217)	154 ± 20 (122, 156, 192)	166 ± 24 (122, 161, 217)
Longest r-r interval (sec)	1.3 ± 0.6 (0.9, 1, 2.4)	1.7 ± 0.6 (0.9, 1.58, 3.10)	2.2 ± 1.2 (1.0, 1.8, 4.3)	1.8 ± 0.9 (0.9, 1.5, 4.3)

Table entries are means ± SD above (minimum, median, maximum).
Details regarding statistical analysis are provided in the Results section.

TABLE 2— Holter Recording With Longest r-r Interval, by *PHOX2B* Genotype From 39 Subjects With CCHS

Characteristic	20/25	20/26	20/27	All 39 cases
Number of subjects	6	16	12	39
Age at longest r-r interval (years)	3.6 ± 1.9 (1.4, 3.0, 6.4)	7.1 ± 6.0 (0.9, 5.2, 19.8)	10.2 ± 5.9 (2.8, 10.7, 19.3)	7.8 ± 5.8 (0.9, 5.8, 19.8)
Minimum heart rate (bpm)	62.8 ± 9.4 (47, 64, 75)	49. 3 ± 10.9 (31, 50, 67)	42.3 ± 7.9 (30, 41, 59)	50.7 ± 14.3 (30, 49, 94)
Mean heart rate (bpm)	105 ± 16 (77, 108, 121)	107 ± 16 (83, 108, 136)	99 ± 15 (78, 95, 120)	104 ± 15 (77, 104, 136)
Maximum heart rate (bpm)	169 ± 23 (138, 169, 194)	168 ± 26 (131, 164, 217)	151 ± 27 (106, 152, 202)	162 ± 26 (106, 158, 217)
Longest r-r interval (sec)	1.3 ± 0.6 (1.0, 1.1, 2.4)	2.4 ± 1.2 (0.9, 2.3, 5.7)	3.8 ± 1.2 (1.7, 4.0, 5.6)	2.7 ± 1.4 (0.9, 2.3, 5.7)

Table entries are means ± SD above (minimum, median, maximum heart rates).
Details regarding statistical analysis are provided in the Results Section.

Holter Recording With Longest r-r Interval

Values for the longest r-r interval by *PHOX2B* genotype are provided in Tables 2 and 3. The longest r-r interval varied by genotype ($\chi^2_{2df} = 13.59$, $P = 0.001$), with *post hoc* comparison showing r-r interval significantly longer for the 20/27 genotype than the 20/25 genotype ($P = 0.002$) and than the 20/26 genotype ($P = 0.01$), and for the 20/26 genotype than the 20/25 genotype ($P = 0.04$). The longest r-r interval was found to be independent of the baseline heart rate as well as the time of day.

To more clearly demonstrate the prolongation of the longest r-r interval among children with CCHS, the data are shown in Table 3 with increasing levels of r-r interval cutoffs by genotype, and in Figure 1. Among children with the 20/25 genotype, only 1 of 6 (16%) has an r-r interval at or above 2 sec and none had an r-r interval at or above

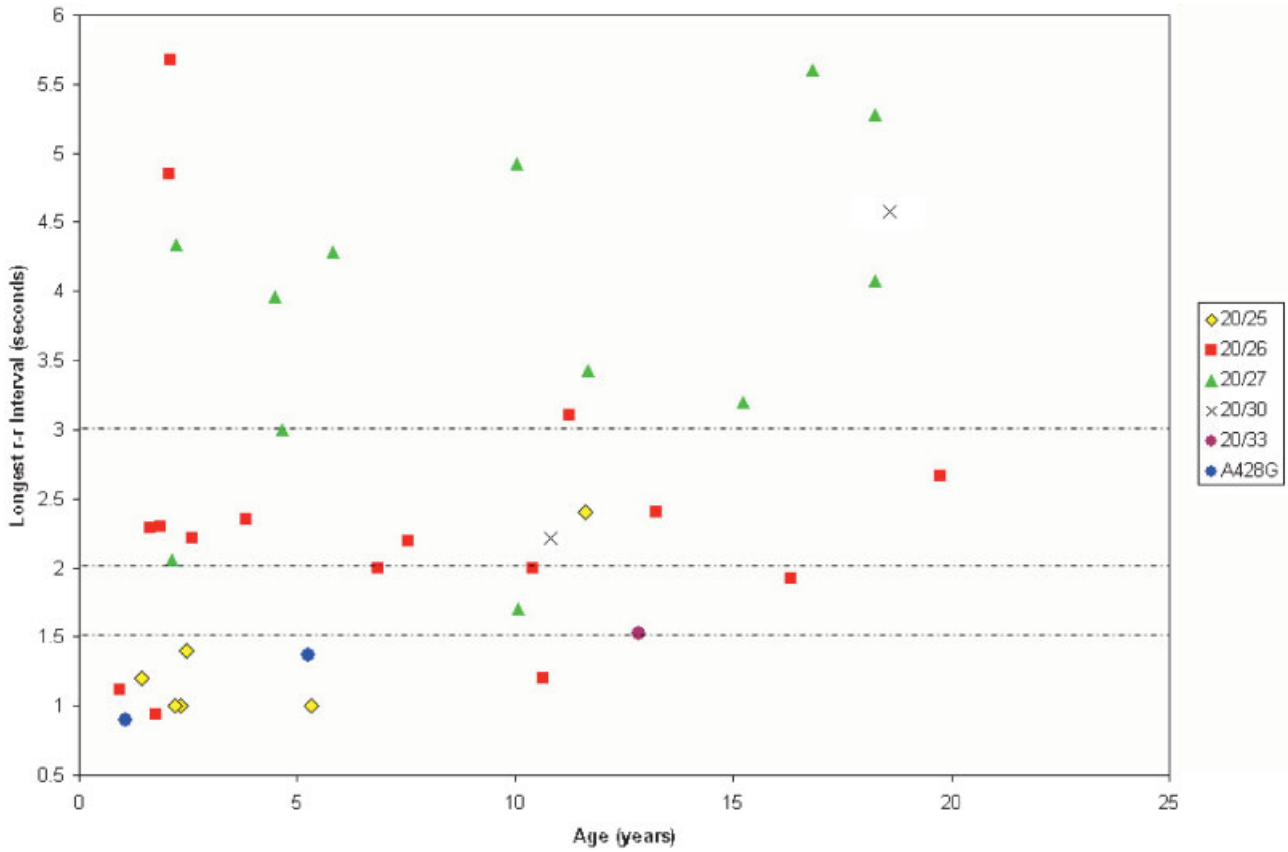

Fig. 1. Longest r-r interval by *PHOX2B* genotype in 39 children with CCHS. This figure demonstrates the longest r-r interval on any Holter recording from the 39 children with CCHS. A horizontal line is drawn at the cutoffs of 1.5, 2.0, and 3.0 sec. As described in the text, there is a positive correlation between the longest r-r interval and the *PHOX2B* genotype within the 20/25, 20/26, and 20/27 comparison. The sample size for the longer polyalanine repeats and the nonpolyalanine repeat mutation were too small for meaningful statistical comparison.

TABLE 3— Longest r-r Interval on Holter Recordings Among 37 Children With CCHS and a Polyalanine Expansion Mutation, by Number of Repeats in *PHOX2B* Genotype

Duration of r-r interval (sec)	20/25	20/26	20/27	Other	Total
<1.5	5	3	0	0	8
1.5–1.99	0	1	1	1	3
2.0–2.99	1	9	1	1	12
≥3.0	0	3	10	1	14
Total	6	16	12	3	37

Details regarding statistical analysis are provided in the Results section.

3 sec. Among children with the 20/26 genotype, 9 of 16 children (56%) have an r-r interval between 2–2.99 sec and 3 of 16 (19%) have an r-r interval of 3 sec or longer. One more child with the 20/26 genotype had a prolonged asystole at age 9, and received a cardiac pacemaker. Because a Holter recording was not performed at the referring hospital to document the asystole, this event is not included in our dataset. Among children with the 20/27 genotype, 1 of 12 children (8%) has an r-r interval between 2–2.99 sec and 10 of 12 (83%) have an r-r interval of 3 sec or longer. These data demonstrate that the proportion of subjects with longest r-r value above the 2.0 sec cutoff is lower for 20/25 than for 20/27 (Fisher's exact test: overall $P = 0.007$ for comparing three groups, 20/25 vs. 20/27: $P = 0.004$, 20/25 vs. 20/26: $P = 0.1$, 20/26 vs. 20/27: $P = 0.2$). Likewise, using a longest r-r cutoff of 3.0 sec, the data indicate a positive association between number of repeats and the number of subjects with a longest r-r value above the cutoff. The proportion of subjects with longest r-r value at or above 3 sec is higher for 20/27 than for 20/25 or 20/26 (Fisher's exact test: three groups $P = 0.002$, 20/25 vs. 20/27: $P = 0.009$, 20/26 vs. 20/27: $P = 0.006$, 20/25 vs. 20/26: $P = 0.5$).

Positive associations between the number of polyalanine repeats and 24 hr/day mechanical ventilation ($\chi^2_1 = 8.46$, $P = 0.004$) and recommendation for diaphragm pacemakers ($\chi^2_1 = 17.36$, $P < 0.0001$) were observed. However, neither pulmonary hypertension nor seizures was associated with the number of polyalanine repeats ($P = 0.1$ and 0.6, respectively).

Long QT Interval Data

Values for the QTc intervals by *PHOX2B* genotype are provided in Table 4 and Figure 2. The maximum QTc interval did not vary by genotype ($\chi^2_{2df} = 0.69$, $P = 0.7$). The QTc was found to be independent of baseline heart rate as well as time of day or night. All subjects with CCHS and scored QTc had at least one Holter with a QTc value greater than the cutoff of 450 msec. The percent of time with QTc values greater than 450 msec were $6.4 \pm 7.0\%$ for 20/25, $28.0 \pm 10.9\%$ for 20/26, $20.7 \pm 30.6\%$ for 20/27, and did not vary by genotype ($\chi^2_{2df} = 4.73$, $P = 0.09$) (Fig. 2).[23,24] The percent of Holter recording with QTc above 450 msec was substantial considering the overall rarity of QTc greater than 440 msec in normal children.

Cardiac Pacemaker Implantation

Thirteen children in the study have ultimately received a cardiac pacemaker (33%), though two had prolonged r-r intervals elsewhere after our study ended. None of the children with the 20/25 genotype received a cardiac pacemaker. Four children with the 20/26 genotype (25%) received a cardiac pacemaker. Eight children with the 20/27 genotype (67%) received a cardiac pacemaker. One child with the 20/30 genotype (50%) received a cardiac pacemaker. The proportion of subjects who received a cardiac pacemaker due to prolonged r-r interval was greater for the children with the 20/27 genotype than the 20/25 or 20/26 genotype (Fisher's exact test: overall $P = 0.01$ for comparing three groups, 20/25 vs. 20/27: $P = 0.01$, 20/25 vs. 20/26: $P = 0.5$, 20/26 vs. 20/27: $P = 0.05$).

Of the 14 patients with an r-r interval of ≥3 sec, three did not receive a cardiac pacemaker. Of these patients, one neurocognitively impaired patient with the 20/26 genotype died suddenly while awake and supported with diaphragm pacing, one patient with the 20/27 genotype is profoundly neurocognitively delayed, and another patient with the 20/27 genotype died in his sleep while being mechanically ventilated.

DISCUSSION

These data represent the first report of an association between the size of the polyalanine repeat expansion

TABLE 4— Corrected QT Interval (QTc) in 16 Subjects With CCHS, by Number of Repeats in *PHOX2B* Genotype

Characteristic	20/25	20/26	20/27	All 16 cases
Number of subjects for QTc	4	5	4	16
Age at QTc (years)	4.6 ± 4.8 (1.4, 2.7, 11.8)	8.0 ± 5.1 (1.9, 10.6, 12.7)	20.7 ± 4.4 (14.8, 20.7, 25.7)	11.4 ± 8.5 (1.1, 11.8, 25.7)
Maximum QTc (msec)	579.5 ± 98.1 (493, 564, 698)	565.6 ± 69.2 (507, 528, 655)	593.5 ± 111.0 (523, 546, 759)	569.1 ± 79.6 (491, 541, 759)
% of recording with QTc >450 msec	6.4 ± 7% (0.2, 5.3, 14.7)	28.0 ± 10.9% (12.8, 30.5, 41.0)	20.7 ± 30.6% (2.0, 7.2, 66.3)	21.9 ± 20.8 (0.2, 13.7, 66.3)

Table entries are means ± SD above (minimum, median, maximum).
Details regarding statistical analysis are provided in the Results section.

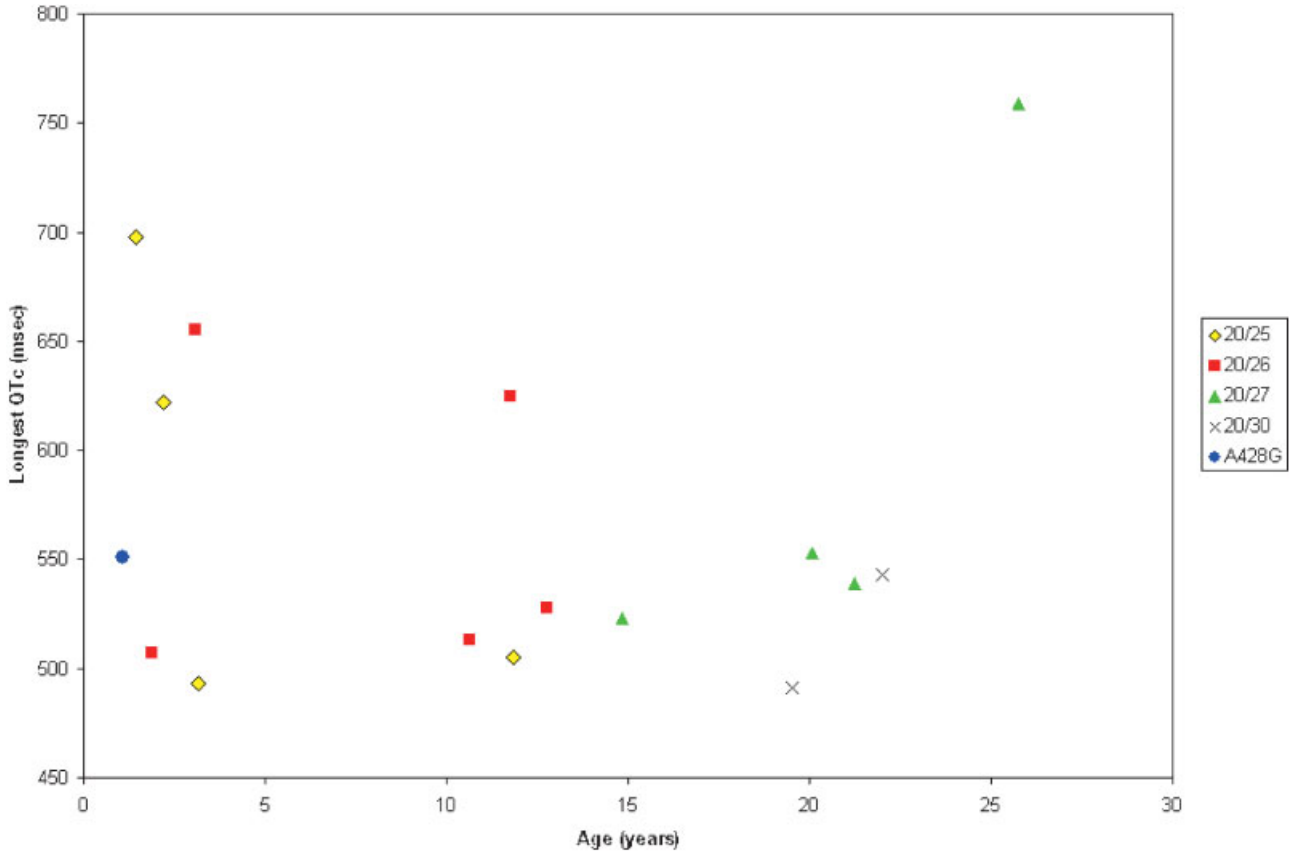

Fig. 2. Longest QTc interval by *PHOX2B* genotype in 16 children with CCHS. This figure demonstrates the longest QTc interval on all Holter recordings for the 16 children in whom QT was measured. All data points for children with CCHS are above the accepted cutoff of 450 msec. Details regarding statistical analysis are provided in the text.

mutation in the CCHS-related *PHOX2B* genotype, the duration of each individual's longest r-r interval on 72-hr Holter recording, and recommendation for cardiac pacemaker implantation. Though longest QTc interval did not vary by genotype, all children with CCHS had at least one Holter with a QTc interval above 450 msec, and the percent of QTc above 450 msec was substantial considering the overall rarity of QTc greater than 440 msec in normal children. These results confirm a disturbance of cardiac autonomic regulation in CCHS and indicate that the *PHOX2B* genotype is related to the severity of dysregulation. Taken together, these results offer the opportunity to identify those individuals with CCHS who may benefit from a cardiac pacemaker and are at the greatest risk of neurocognitive impairment and sudden death.

These findings follow logically from the role of *PHOX2B* in the development of the autonomic nervous system. *PHOX2B* is expressed embryologically in the seventh and ninth/tenth ganglia at day 32 of development. At day 33, *PHOX2B* is expressed in terminal rhombomeres 4–8, in the presumptive enteric ganglia, the sympathetic chain ganglia, and the presumptive carotid body at the carotid bifurcation.[6] In *PHOX2B*-knockout mice the carotid body and autonomic visceral sensory ganglia, including the geniculate, petrosal, and nodose ganglia, fail to form properly and degenerate, and the solitary tract nucleus, their central target which integrates all visceral information including cardiorespiratory regulation, never develops.[25,26] This lack of visceral input may help explain the distinctive lack of flexibility of the cardiovascular system in children with CCHS, thereby increasing their vulnerability to sudden death. These knockout studies demonstrate the importance of *PHOX2B* in the development of the ANS, and suggest a mechanism by which a *PHOX2B* mutation might manifest as dysregulation of cardiac and respiratory rate and rhythm.

The occurrence of prolonged r-r interval in individuals with CCHS, particularly in those subjects with the genotypes of 20/26 and 20/27, is considered to be a risk for sudden death. Though some children may demonstrate staring spells or syncope at the time of the transient asystole, most subjects are asymptomatic. This underlying propensity for prolonged r-r interval may increase the vulnerability of the child with CCHS when exposed to pharmacologic agents or anesthesia. A 9-year-old child

with CCHS was reported to develop a complete atrioventricular heart block after receiving propofol for induction of anesthesia for strabismus surgery.[27] Propofol reduces sympathetic activity and further potentiates vagal stimulation, actions that might heighten cardiovascular dysregulation in the child with CCHS. Likewise, the recent report of death among adolescents with CCHS after involvement in alcohol and substance abuse[28] raises the possibility of an effect on an underlying cardiac vulnerability in addition to the anticipated respiratory compromise. These adverse outcomes in children with CCHS highlight the importance of knowing the baseline r-r intervals among these vulnerable children. In our study, only 11 of the 14 children with longest r-r intervals of 3 sec or longer received a cardiac pacemaker (79%). Of the three remaining patients, one neurocognitively impaired patient with the 20/26 genotype died suddenly while awake, one patient with the 20/27 genotype is profoundly neurocognitively delayed, and another patient with the 20/27 genotype died in his sleep while being mechanically ventilated. The reticence to implant a cardiac pacemaker may be the result of uncertainty as to the clinical significance of prolonged r-r intervals in the seemingly asymptomatic subject, particularly if subsequent r-r intervals are shorter than 3 sec. Because the impact of prolonged sinus pauses on long term neurodevelopment is unknown, and because childhood is such a critical period for neurocognitive development coupled with the overriding risk for sudden death, recommendation for implantation of a cardiac pacemaker in the child with CCHS and r-r intervals of 3 sec or longer seems to be the most conservative recommendation.

Prolonged QTc intervals have been associated with increased risk of malignant arrhythmias and sudden death.[24,29,30] Data from the International Long QT Syndrome Registry indicate that among 1,496 enrolled subjects, the hazard ratio for cardiac events (arrhythmic syncope or probably long QT related death) is 1.052^{x}, where x is the per 10 msec unit increase in QTc.[24] A subject with a QTc of 450 msec therefore would have a 1.29-fold greater risk of a cardiac event than a subject with a QTc of 400 msec ($1.052^{5} = 1.29$). There are several formulas used to convert the measured QTc to a value corrected for the heart rate, but the Bazett formula, $QTc = QT/RR^{1/2}$, has been most frequently used in medical publications and therefore most reported criteria for normal and abnormal values are derived from this formula.[23] Suggested QTc cutoffs for children age 1–15 years of either gender, which includes most subjects of our study, are the following: <440 msec is rated as normal, 440–460 msec as borderline, and >460 msec as prolonged. For adult males, the borderline between normal and prolonged ranges from 430 to 450, and for adult females, the borderline range is 450–470.[23,24] In a study of 581 healthy individuals, including 158 children, 5.7% of children were found to have QTc intervals greater than 440 msec, no adult men had QTc intervals greater than 440 msec, and 8.6% of adult women had values greater than 440.[24] These data make our observation that 100% of children with CCHS for whom QTc intervals were available had values greater than the more stringent 450 msec cutoff used in our study even more remarkable.

Although our results indicating a relationship between *PHOX2B* genotype and r-r and QTc intervals are compelling, they should be interpreted with caution due to potential limitations of our study. First, the distribution of the *PHOX2B* genotypes of subjects enrolled in the study is a possible limitation, with the majority of subjects having the 20/26 and 20/27 genotypes and fewer children having the 20/25 genotype or genotypes of 20/28 to 20/33. It is possible that a greater representation of the less common genotypes may have modified our results. A second limitation is the potential for diaphragm pacer-induced artifact on the ECG channel that might shorten the calculated longest r-r interval (the diaphragm pacer artifact might be misinterpreted as an r wave by computerized analysis). Since diaphragm pacers are most common in the children who are typically dependent on artificial ventilation 24 hr/day and who have the longest polyalanine repeat expansions, this potential underestimation of the longest r-r interval would disproportionately affect these children. As a result, this confounding variable may obscure an even more striking *PHOX2B* genotype/longest r-r phenotype relationship by making the r-r interval appear shorter due to the pacer-induced artifact. Third, the relatively brief and sporadic 72 hr in-hospital Holter recording duration is a limitation of the study. These children were generally observed for three consecutive days at yearly intervals. Due to the unpredictability and transience of prolonged r-r intervals, it is possible that this small period of in-hospital observation resulted in an underestimation of the frequency and length of the sinus pauses documented in CCHS. Taken together, the effect of these limitations may be that our results underestimate the severity of these findings in children with CCHS.

This study has implications for the clinical management of patients with CCHS. Our data indicate that patients with *PHOX2B* genotypes of 20/26 and 20/27 are at an increased risk of prolonged asystole, and need to undergo prolonged Holter recordings as part of their routine clinical care to monitor for these events. Exposure to pharmacologic agents that might impact cardiac rhythm should be avoided. Superficially, patients with the 20/25 genotype do not appear to need regular Holter recordings. However, an isolated report of prolonged r-r intervals in an adult-onset patient [5] indicates the possibility of patients with even the 20/25 genotype exhibiting prolonged r-r intervals in adulthood. Likewise, the sudden death of two of our patients in early adulthood suggests the need for continued

surveillance for prolonged r-r intervals with Holter recordings and the strong recommendation for cardiac pacemaker implantation with r-r intervals $\geq$3 sec, even if subsequent Holter data do not indicate prolonged r-r intervals. Thus it remains within conservative management to continue with Holter monitoring in individuals with CCHS until the impact of adulthood on genotype can be determined. The identified limitations might be addressed by increased Holter monitoring duration and frequency, and possibly in-home recordings. In so doing, more frequent and severe sinus pauses may be documented and a pattern to the pauses might be identified. Long-term neurodevelopmental studies on children with CCHS may help characterize the long-term correlates of the asystoles experienced by these children. In so doing we can optimize the clinical information needed to assure the safety of these vulnerable children and young adults with CCHS and ANS dysregulation, and in so doing avert potential sudden death and/or neurocognitive compromise due to asystole-induced decreases in cerebral perfusion.

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REFERENCES

1. Axelrod FB, Chelimsky GG, Weese-Mayer DE. Pediatric autonomic disorders. Pediatrics 2006;18:309–321.
2. Weese-Mayer DE, Shannon DC, Keens TG, Silvestri JM. Idiopathic congenital central hypoventilation syndrome: diagnosis and management. American Thoracic Society. Am J Respir Crit Care Med 1999;160:368–373.
3. Todd ES, Weinberg SM, Berry-Kravis EM, Silvestri JM, Kenny AS, Rand CM, Zhou L, Maher BS, Marazita ML, Weese-Mayer DE. Facial phenotype in children and young adults with *PHOX2B*-determined congenital central hypoventilation syndrome: quantitative pattern of dysmorphology. Pediatr Res 2006;59:39–45.
4. Weese-Mayer DE, Berry-Kravis EM, Zhou L. Adult identified with CCHS-mutation in *PHOX2B* gene and late onset CHS. Am J Respir Crit Care Med 2005;171:88.
5. Antic NA, Malow BA, Lange N, McEvoy RD, Olson AL, Turkington P, Windisch W, Samuels M, Stevens CA, Berry-Kravis EM, Weese-Mayer DE. *PHOX2B* mutation-confirmed congenital central hypoventilation syndrome: presentation in adulthood. Am J Respir Crit Care Med 2006;174:923–927.
6. Amiel J, Laudier B, Attie-Bitach T, Trang H, de Pontual L, Gener B, Trochet D, Etchevers H, Ray P. Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. Nat Genet 2003;33:1–3.
7. Weese-Mayer DE, Berry-Kravis EM, Zhou L, Maher BS, Silvestri JM, Curran ME, Marazita ML. Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in *PHOX2B*. Am J Med Genet 2003; 123A:267–278.
8. Sasaki A, Kanai M, Kijima K, Akaba K, Hashimoto M, Hasegawa H, Otaki S, Koizumi T, Kusuda S, Ogawa Y, Tuchiya K, Yamamoto W, Nakamura T, Hayasaka K. Molecular analysis of congenital central hypoventilation syndrome. Hum Genet 2003; 114:22–26.
9. Matera I, Bachetti T, Puppo F, Di Duca M, Morandi F, Casiraghi GM, Cilio MR, Hennekam R, Hofstra R, Schober JG, Ravazzolo R, Ottonello G, Ceccherini I. *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated systems in both congenital and late onset central hypoventilation syndrome. J Med Genet 2004;41: 373–380.
10. Berry-Kravis EM, Zhou L, Rand CM, Weese-Mayer DE. Congenital central hypoventilation syndrome: *PHOX2B* mutations and phenotype. Am J Respir Crit Care Med 2006;174:1139–1144.
11. Trochet D, O'Brien LM, Gozal D, Trang H, Nordenskjold A, Laudier B, Svensson PJ, Uhrig S, Cole T, Niemann S, Munnich A, Gaultier C, Lyonnet S, Amiel J. *PHOX2B* genotype allows for prediction of tumor risk in congenital central hypoventilation syndrome. Am J Hum Genet 2005;76:421–426.
12. Woo MS, Woo MA, Gozal D, Jansen MT, Keens TG, Harper RM. Heart rate variability in congenital central hypoventilation syndrome. Pediatr Res 1992;31:291–296.
13. Shea SA, Andres LP, Paydarfar D, Banzett RB, Shannon DC. Effect of mental activity on breathing in congenital central hypoventilation syndrome. Respir Physiol 1993;94:251–263.
14. Trang H, Boureghda S, Denjoy I, Alia M, Kabaker M. 24-hr BP in children with congenital central hypoventilation syndrome. Chest 2003;124:1393–1399.
15. Silvestri JM, Weese-Mayer DE, Flanagan EA. Congenital central hypoventilation syndrome: cardiorespiratory responses to moderate exercise, simulating daily activity. Pediatr Pulmonol 1995; 20:89–93.
16. Silvestri JM, Hanna BD, Volgman AS, Jones PJ, Barnes SD, Weese-Mayer DE. Cardiac rhythm disturbances among children with idiopathic congenital central hypoventilation syndrome. Pediatr Pulmonol 2000;29:351–358.
17. Trang H, Girard A, Laude D, Elghozi J. Short-term blood pressure and heart rate variability in congenital central hypoventilation syndrome (Ondine's Curse). Clin Sci 2005;108:225–230.
18. Ramanathan R, Corwin MJ, Hunt CE, Lister G, Tinsley LR, Baird T, Silvestri JM, Crowell DH, Hufford D, Martin RJ, Neuman MR, Weese-Mayer DE, Cupples LA, Peucker M, Willinger M, Keens TG. Cardiorespiratory events recorded on home monitors: comparison of healthy infants with those at increased risk for SIDS. JAMA 2001;285:2199–2207.
19. Southall DP, Richards J, Mitchell P, Brown DJ, Johnston PGB, Shinebourn EA. Study of cardiac rhythm in healthy newborn infants. Br Heart J 1980;43:14–20.
20. Southall DP, Johnston F, Shinebourne EA, Johnston PG. 24-hour electrocardiographic study of heart rate and rhythm patterns in population of healthy children. Br Heart J 1981;45:281–291.
21. Nagashima M, Matsushima M, Ogawa A, Ohsuga A, Kaneko T, Yazaki T, Okajima M. Cardiac arrhythmias in healthy children revealed by 24-hour ambulatory ECG monitoring. Pediatr Cardiol 1987;8:103–108.
22. Viitasalo MT, Kala R, Eisalo A. Ambulatory electrocardiographic recording in endurance athletes. Br Heart J 1982;47:213–220.
23. Goldenberg I, Moss AJ, Zareba W. QT interval: how to measure it and what is ''normal''. J Cardiovasc Electrophys 2006;17:333–336.

24. Moss AJ. Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. Am J Cardiol 1993;72: 23B–25B.
25. Pattyn A, Morin X, Cremer H, Goridis C, Brunet J. The homeobox gene *PHOX2B* is essential for the development of autonomic neural crest derivatives. Nature 1999;399:366–370.
26. Dauger S, Pattyn A, Lofaso F, Gaultier C, Goridis C, Gallego J, Brunet JF. *PHOX2B* controls the development of peripheral chemoreceptors and afferent visceral pathways. Development 2003;130:6635–6642.
27. Sochala C, van Deenen D, de Ville A, Govaerts MJM. Heart block following propofol in a child. Paediatr Anaesth 1999;9:349–351.
28. Chen ML, Turkel SB, Jacobson JR, Keens TG. Alcohol use in congenital central hypoventilation syndrome. Pediatr Pulmonol 2006;41:283–285.
29. Sauer AJ, Moss AJ, McNitt S, Peterson DR, Zareba W, Robinson JL, Qi M, Goldenberg I, Hobbs JB, Ackerman MJ, Benhorin J, Hall WJ, Kaufman ES, Locati EH, Napolitano C, Priori SG, Schwartz PJ, Towbin JA, Vincent GM, Zhang L. Long QT syndrome in adults. J Am Coll Cardiol 2007;49:329–337.
30. Tester DJ, Will ML, Haglund CM, Ackerman MJ. Effect of clinical phenotype on yield of long QT syndrome genetic testing. J Am Coll Cardiol 2006;47:764–768.