Autonomic Function in Children With Congenital Central Hypoventilation Syndrome and Their Families*

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Study objectives: Congenital central hypoventilation syndrome (CCHS) is a genetic disorder characterized by failure of automatic control of breathing in the absence of obvious anatomic lesions. There have been several reports suggesting that CCHS patients display autonomic dysregulation. Pulse arterial tonometry (PAT) is a novel technique that provides noninvasive moment-to-moment measurements of sympathetic tone changes to the cutaneous vascular bed. We hypothesized that autonomic function as measured by PAT would be altered in children with CCHS.

Design: Prospective study.

Setting: CCHS Family Conference, Orlando, FL, and the local community in Louisville, KY.

Participants: Nineteen CCHS patients and 31 parents as well as 24 control children and 15 adult control subjects.

Interventions: Children with CCHS and their parents underwent sympathetic challenges (vital capacity sigh and cold hand pressor test) and a test of reactive hyperemia (brachial artery occlusion) while PAT was continuously monitored from the right hand. Control children and control adults underwent the same procedure.

Measurements and results: The maximal change of the PAT signal compared to the preceding baseline was averaged and expressed as percentage change for each of the challenges. The magnitude of sympathetic discharge-induced attenuation of PAT signal following a sigh was reduced in CCHS children compared to control subjects for both the vital capacity sighs and the cold hand pressor test. There were no differences observed in the magnitude of PAT attenuation between parents of children with CCHS and control adults. No differences were observed between either CCHS and control subjects or CCHS parents and adult control subjects for the brachial artery occlusion test.

Conclusion: CCHS patients show an attenuated response to endogenous sympathetic stimulation, supporting the presence of autonomic nervous system dysfunction as a consistent feature of this condition. No differences were found in parents of children with CCHS compared to control adults, consistent with the finding that CCHS is primarily the result of a de novo gene mutation.

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Key words: autonomic nervous system dysfunction; congenital central hypoventilation syndrome; pulse arterial tonometry

Abbreviations: ANS = autonomic nervous system; CCHS = congenital central hypoventilation syndrome; CI = confidence interval; NS = not significant; PAT = pulse arterial tonometry

Congenital central hypoventilation syndrome (CCHS) is a rare condition characterized by failure of automatic control of breathing in the absence of obvious anatomic lesions. One of the primary manifestations of CCHS consists of alveolar hypoventilation and diminished tidal volume that are more pronounced during sleep than during wakefulness, and are most affected during slow wave sleep.
While CCHS was originally considered a disorder of ventilatory control, increasing cumulative evidence suggests that this disorder is characterized by a more generalized involvement of the autonomic nervous system (ANS).4 Indeed, decreased heart rate variability and increased ratios of low-frequency/high-frequency spectral power bands were found in 12 CCHS patients compared to matched control subjects,5 a finding that strongly suggested the presence of imbalance in sympathetic/parasympathetic regulation. Other reports revealing abnormal development of neural crest-derived cells,6–9 cardiac rhythm disturbances,10 abnormal esophageal motility,2,11 and multiple ocular problems12,13 further reinforce the concept that ANS dysfunction is universally present in CCHS patients.

A genetic basis for CCHS has long been suspected based on familial cases,6,14 associations with Hirschsprung disease,2,6,7 vertical transmission,15,16 and high prevalence of reported symptoms of ANS dysfunction in families.17,18 Research in mice has revealed that development of ANS reflex circuits is critically dependent on the paired-like homeobox gene Phox2b19 and that Phox2b/- mice die in utero with absent ANS circuits.20 These initial discoveries led to identification of mutations in the human PHOX2B gene as the primary suspect in CCHS characteristics.21 Furthermore, mutations in PHOX2B gene followed an autosomal-dominant mode of inheritance with de novo mutation at the first generation,21 and with a heterozygous mutation being identified in 92.5% of cases.22 These genetic studies are in conflict with the previous reports on a higher prevalence of ANS dysfunction in large cohorts of families of CCHS patients,17,18 since de novo mutations would imply normal ANS function in the parents of children born with CCHS.

Development of a novel technique, pulse arterial tonometry (PAT) [Itamar Medical, Caesarea, Israel],23 which allows for simple noninvasive measurement of sympathetic discharge to the digital vascular bed prompted us to explore whether patients with CCHS and their parents do exhibit abnormal ANS responses. PAT employs a plethysmographic approach that continuously measures the arterial pulsatile volume change while reducing contributions from venous pulsations. Changes in sympathetic nervous system activity in the cutaneous vasculature, which are mediated by α-adrenoreceptor activation, result in episodic vasoconstriction of the digital vascular beds with corresponding attenuation of PAT signal.24,25 Thus, increases in peripheral sympathetic activity will elicit robust changes in peripheral vascular cutaneous perfusion and can be readily detected on a beat-to-beat basis. PAT measurements allow therefore for relatively simple assessments of sympathetic activity responses in both adults23 and in children.26,27

The major aims of this study were to investigate the dynamic responses of the sympathetic ANS in children with CCHS and their parents and to compare these responses to control children and adults using standard stimuli coupled to PAT measurements. We hypothesized that children with CCHS would have altered autonomic function while, based on genetic evidence, no such alterations would be apparent in their parents. Furthermore, because the PAT technique has also been shown to reliably assess peripheral microvascular endothelial function in response to reactive hyperemia,28,29 we further wished to assess whether endothelial function would be altered in children with CCHS following a short period of induced arterial occlusion of the upper extremity.

Materials and Methods

Children with CCHS and their parents were recruited during the CCHS Family Conference in Orlando, FL, in July 2003. Control children and adults were recruited from the local population in Louisville, KY. This study was approved by the Institutional Review Board at the University of Louisville. Informed consent and child assent in the presence of a parent were obtained for all participants.

PAT was used to assess changes in sympathetic activity during quiet wakefulness. The PAT probe was placed on the first finger of the right hand of each subject, and both raw and filtered PAT signals were digitally acquired. Each subject sat up straight with both hands resting on a table in front of them. Baseline PAT measurements were obtained when subjects were sitting quietly and the PAT signal was stable for at least 2 min. Baseline values were obtained by taking the mean measurement across a 10-s period of stable, artifact-free signal. Following baseline measures, each subject underwent a series of four vital capacity sighs, each separated by at least 60 s of normal breathing wherein the PAT signal remained stable and artifact free. The maximal attenuation of PAT signal was measured over 10 s and calculated as percentage change from baseline. Measurements were averaged over the series of sighs.

Five minutes after the sigh maneuvers and after the PAT signal had returned to a steady state, a BP cuff was placed around the upper right arm of each subject and manually inflated to suprasystolic pressure. The resultant brachial artery occlusion was maintained for 60 s and then released, with ensuing reactive hyperemia being recorded from the PAT device. The extent of reactive hyperemia was derived from the mean PAT amplitude over a period of 30 s, starting 30 s after the release of the occlusion. These changes were then normalized as a percentage of the baseline measurements sampled over a period of 30 s preceding the brachial occlusion maneuver.

Finally, at least 5 min after the cuff occlusion, during which baseline PAT values were stable, subjects underwent a cold hand pressor test. The left hand was immersed in ice-cold water (approximately 3°C) for 30 s while the right hand remained still. PAT signal was continuously monitored throughout the immersion and recovery periods in the right hand. Changes in signal amplitude were calculated by averaging the mean amplitude over
a 10-s period before the pressor test (baseline), during the pressor test (challenge), immediately following the removal of the hand from the water (postchallenge), and then at 1-min intervals during the 5-min recovery phase. All measures were obtained from a steady and regular artifact-free PAT signal. Amplitudes were expressed as percentage change from the corresponding baseline.

Data Analysis

All data sets were numerically coded and analyzed blind to the subject group on completion of recruitment. Data are presented as means ± SD and 95% confidence intervals (CIs) unless otherwise indicated. Independent t tests were employed for comparisons of measures between the study groups, with a Fisher exact test used for dichotomous outcomes. For the cold hand pressor tests, analysis of variance with repeated measures was employed. All p values reported are two sided, with statistical significance set at p < 0.05.

Results

Nineteen children with CCHS were studied (10 boys; mean age, 12 ± 4.2 years) together with 31 CCHS parents (14 male adults). Twenty-four control children were also studied (15 boys; mean age, 10 ± 3.3 years) as well as 15 control adults (7 male adults).

Phenotype/Genotype Characteristics

Phenotypic characteristics were available for all children with CCHS and are given in Table 1. Genotype results were available for 17 of the 19 CCHS children and 28 of the 31 parents. All 17 CCHS children for whom genotype results were available, with the exception of 1 child, were found to have a polyalanine mutation in the PHOX2B gene, while in only 1 parent somatic mosaicism of PHOX2B was found.

Sigh Maneuvers

Sympathetic-induced attenuation of the PAT signal following a voluntary vital capacity sigh is illustrated in Figure 1. Control children showed significantly greater attenuation than children with CCHS; mean attenuation from baseline was 56.6 ± 13% (95% CI, 51.4 to 61.8%) in control children, compared to 34.4 ± 16.1% in CCHS children (95% CI, 27.2 to 41.6%; p < 0.0001) [Fig 2]. Furthermore, 74% of children with CCHS had PAT attenuations > 1 SD from the mean of the control values, while 37% of children with CCHS had PAT attenuations > 2 SDs from the mean of the control values. There were no differences observed in the magnitude of PAT attenuation between parents of children with CCHS and control adults (mean attenuation, 42.4 ± 15.8%; 95% CI, 36.8 to 48.0; and 38.4 ± 24.7%; 95% CI, 25.9 to 50.9, respectively; p = not significant [NS]).

Table 1—Main Phenotypic Characteristics of the Children With CCHS (n = 19)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Children, No. (%)</th>
<th>Male Children, No.</th>
</tr>
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<tbody>
<tr>
<td>Hirschsprung disease</td>
<td>2 (10.5)</td>
<td>1</td>
</tr>
<tr>
<td>Heart rate disorders</td>
<td>4 (21)</td>
<td>1</td>
</tr>
<tr>
<td>Neurologic disorders</td>
<td>2 (10.5)</td>
<td>0</td>
</tr>
<tr>
<td>Ocular disorders</td>
<td>9 (47)</td>
<td>4</td>
</tr>
<tr>
<td>Obesity</td>
<td>2 (10.5)</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1. PAT attenuation associated with a vital capacity sigh in a control child (top, A) and a child with CCHS (bottom, B).

Figure 2. Mean and 95% CI for PAT attenuation during vital capacity sighs in children with CCHS and control subjects.
Cuff Occlusion

The mean magnitude of reactive hyperemia measured by the PAT signal following brachial artery occlusion is illustrated in Figure 3. Control children showed greater hyperemia than children with CCHS, although this did not reach statistical significance (Fig 3). There was significantly greater variability in the vascular hyperemic responses among control subjects compared to CCHS. Indeed, the mean change from baseline was 31.0 ± 61.5% (95% CI, −5.3 to 67.3%) in control subjects, compared to 6.1 ± 21.2% in CCHS children (95% CI −3.7 to 15.9%; p = 0.13 [NS]). Furthermore, 44% of children with CCHS did not demonstrate a typical overshoot pattern, compared to 18% of control children, although this did not reach statistical significance (p = 0.29, NS). There were no differences observed in the magnitude of PAT-measured reactive hyperemia between parents of children with CCHS and control adults.

Cold Hand Pressor Test

The magnitude of sympathetic-induced attenuation of the PAT signal during the cold hand pressor test showed that control children had greater attenuation than children with CCHS (77.0 ± 12.0% [95% CI, 71.6 to 82.4%] in control children, compared to 53.0 ± 19.0% [95% CI, 44.0 to 62.0%] in children with CCHS, respectively; p < 0.0001). Furthermore, the recovery dynamics of the children with CCHS were significantly faster than those of control children, with a return to baseline within 1 min, compared to 4 min for control children (p < 0.001).

No differences in mean attenuation during the cold hand pressor test or recovery dynamics were observed between parents of children with CCHS and control adults. (mean attenuation, 47.6 ± 19.7%; 95% CI, 40.4 to 54.8; and 53.0 ± 18.5%; 95% CI, 43.3 to 62.7, respectively; p = NS). Of note, no differences were observed between male and female subjects for any of the challenges. Furthermore, no relationship was found between the amplitude of responses and clinical symptoms for the CCHS children.

Discussion

This study shows that children with CCHS have attenuated peripheral responses to sympathetic stimulation, thereby further confirming the presence of ANS dysfunction as a consistent feature of this genetic condition. Moreover, a spectrum of ANS dysfunction was present, with over a third of children with CCHS showing mean attenuations of the PAT signal following vital capacity sighs that were > 2 SDs from control values. The observation that no differences were found between the autonomic responses of the parents of children with CCHS and healthy control adults is consistent with the findings of Amiel and colleagues, indicating that CCHS is primarily the result of de novo mutations, rather than reflect transmission of existing mutations, the latter being found in a minority of patients. Finally, using the brachial occlusion test, we found no evidence to support the presence of significant alterations in endothelial function in children with CCHS.

In the present study, we used PAT, a novel technique for noninvasive assessment of moment-to-moment changes in peripheral sympathetic vasoconstrictor tone. Increases in sympathetic tone were readily identified with this technique by the occurrence of a dose-dependent attenuation of the PAT signal, such that greater sympathetic activity surges will result in greater PAT signal attenuation. We selected two simple stimulation paradigms, namely vital capacity sighs and the cold pressor test. Such now classic tests have been shown to induce an α-adrenoceptor–mediated vasoconstriction in the peripheral vasculature, which is sympathetically mediated and can be blocked by pretreatment with α-adrenoceptor antagonists.

We found attenuated sympathetic responses in a large proportion of children with CCHS compared to control children. In contrast, no differences in sympathetic tone emerged among the parents of CCHS children when compared to adult control subjects. This finding is consistent with the genotype studies of Amiel et al. who showed that the PHOX2B gene is the primary gene underlying CCHS with a de novo mutation pattern at the first generation as the leading presentation of this com-
plex genetic disorder. However, our findings contrast with those of Weese-Mayer and colleagues,18 who reported on the high prevalence of symptoms compatible with ANS dysfunction in a epidemiologic survey of a large case-control study of children with CCHS and their relatives. This study interviewed 56 CCHS patients and 56 age- and gender-matched control subjects as well as multiple family members encompassing three generations and a total of 2,353 subjects for symptoms suggestive of ANS problems. Such symptoms were more likely in relatives of CCHS patients than in relatives of control subjects and tended to be present with milder degrees of severity in non-CCHS relatives. Furthermore, segregation analysis suggested that the pattern of transmission was consistent with Mendelian transmission.17 While we did not find any differences in ANS function using PAT in the parents of CCHS children compared to control adults, it is possible that very small differences may exist and that our relatively small sample size did not allow for their detection. Indeed, while the negative findings among parents of CCHS children are consistent with those of Amiel et al21 showing a de novo mutation at the first generation, a subsequent study22 on a larger sample of children with CCHS (n = 188) found that somatic masochism was detected in 10 of all parents studied (4.5%). Thus, it remains possible that a small proportion, most likely approximating 5%, of parents of CCHS children may have subtle alterations of ANS function. We could not find any correlation between the specific characteristics of the gene mutation (eg, the number of polyalanine expansions) and the magnitude of ANS dysfunction in our patients. This is not surprising, considering the absence of such relationship for other phenotypic features of CCHS.33

Further evidence for alterations in ANS function have been suggested by abnormal circadian BP patterns observed in 11 children with CCHS and 11 matched control subjects.34 Children with CCHS were found to have lower BP during wakefulness and higher BP during sleep, with abnormally reduced nocturnal dipping. Indeed, 91% of CCHS were non-dippers, compared with none of the control subjects. Such abnormalities may be related to abnormalities in central autonomic regulation, since the control of BP and heart rate is mediated by the balance of parasympathetic and sympathetic activity. Recently, Trang et al35 have provided new insights into the pathophysiology of CCHS by evaluating heart rate variability and BP changes in 12 children with CCHS requiring nightly ventilatory assistance. Continuous BP measurements were obtained during wakefulness in the supine, head-up tilt, and standing positions. BP levels were maintained at rest; however, a limited capacity to elevate BP during ortho-

static maneuvers emerged, with absence of the BP overshoot typically seen during active standing also being noted. The consistent reduction in heart rate variability found in patients with CCHS during the circadian cycle or during specific challenges such as ventilatory stimulation5,35,36 as well as the decreased baroreflex sensitivity suggest that there is a predominant vagal dysfunction with a relative preservation of sympathetic function, further highlighting the broad and complex nature of ANS dysfunction observed in this condition. The discrepancy between the latter findings of predominant vagal dysfunction and our findings of sympathetic dysfunction may be due, in part, to the index of sympathetic outflow measured. The PAT device measures peripheral vascular tone, whereas baroreceptor afferents do not regulate sympathetic vasoconstrictor outflow to the cutaneous circulation.37 Furthermore, body positional differences may modify both cardiovascular and respiratory response characteristics, which is of particular relevance to BP measurements since these are closely related to vestibular influences and other postural mechanisms.38

The aberrant cold pressor responses have also been recently assessed using a forehead cooling paradigm with parallel functional MRI techniques.39 This study revealed deficiencies in activation of brain regions that are responsible for the integration of cold afferent stimulation with respiratory and cardiovascular output, particularly dorsal medulla, cerebellar cortex and deep nuclei, basal ganglia, and middle-to-posterior cingulate, insular, frontal, and temporal cortices. We postulate that the extensive CNS involvement imposed by the abnormal development of ANS structures as dictated by the genetic mutations associated with CCHS is counterbalanced, at least partially, by existing redundancies and plasticity in the regulatory systems governing ANS function. In other words, the ability of a neural region underlying ANS functional regulatory properties to take over a particular function when another region(s) is affected by the disease condition may clearly lead to palliation in the severity of the phenotypic manifestation measured. The extent and efficacy of such redundant systems in overcoming the structural ANS deficits will thus dictate the variability of phenotypic expression of ANS dysfunction identified in this and other studies.

In addition to the measures of sympathetic tone that were obtained in the current study with the PAT device, we also conducted explorative studies on endothelial function in CCHS by means of temporary brachial artery occlusion approaches. Indeed, the postocclusive vascular reactivity test enables measurement of endothelium-dependent vasodilation.40 While brachial ultrasound is the “gold stan-

2482 Clinical Investigations
dard” for measuring reactive hyperemia, it is fraught with technical difficulties that limit its widespread applicability.41 PAT signal analysis provides a reliable surrogate for measurement of endothelial function and has been shown to correlate with the results of brachial ultrasound.29 We did not find significant differences in endothelial function among CCHS children and control subjects. However, control children displayed a tendency toward larger hyperemic response as well as a larger variability in their responses, with almost half of the children with CCHS not demonstrating the expected overshoot in regional blood flow. While these results did not reach statistical significance, it is possible that differences in endothelial function might still be present but that the relatively small sample size was underpowered to detect such alterations. This is an area that warrants further investigation.

In summary, we have shown that dynamic sympathetic tone responses are attenuated in children with CCHS compared to healthy control subjects. Moreover, we were unable to detect any differences in sympathetic tone responses in the parents of these children when compared to control subjects. These findings are in line with the recent genetic data22 of a de novo mutation accounting for most CCHS cases.

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