



ORIGINAL ARTICLE

Inheritance of polyalanine expansion mutation of *PHOX2B* in congenital central hypoventilation syndrome

Toru Meguro¹, Yuki Yoshida¹, Makiko Hayashi¹, Kentaro Toyota¹, Tesshu Otagiri¹, Narutaka Mochizuki², Yumiko Kishikawa¹, Ayako Sasaki¹ and Kiyoshi Hayasaka¹

Congenital central hypoventilation syndrome (CCHS; MIM 209880) is caused mostly by dominant alanine expansion (most prevalent is 7-alanine expansion) mutations in *PHOX2B*. More than 90% of the alanine expansion mutations had been considered to be *de novo* due to unequal crossover during gametogenesis. However, a recent report stated that 25% of patients inherited the alanine-expanded allele from their parents with somatic mosaicism or constitutive mutation. We studied inheritance in 45 unrelated families, and found that one patient (2%) inherited 5-alanine expansion mutation from a parent with late-onset central hypoventilation syndrome and nine patients (20%) inherited 5- to 7-alanine expansion mutation from apparently asymptomatic parents with somatic mosaicism. Analysis using a sensitive method would be recommended to all parents of CCHS proband due to high incidence of somatic mosaicism. The absence of an alanine-contracted allele (expected counterpart allele in unequal crossover) and the highest prevalence of 6-alanine expansion mutation in somatic mosaicism suggest that the somatic mosaicism is likely caused by a mechanism other than an unequal crossover, such as a replication mechanism.

Journal of Human Genetics (2012) 57, 335–337; doi:10.1038/jhg.2012.27; published online 22 March 2012

Keywords: congenital central hypoventilation syndrome; mosaic; *PHOX2B*; polyalanine

INTRODUCTION

Congenital central hypoventilation syndrome (CCHS; MIM 209880) is characterized by failure of the automatic control of breathing during sleep, and results from the dominant *PHOX2B* mutation. More than 90% of patients have polyalanine expansion mutations in the polyaniline tract of 20 residues, and less than 10% of patients have frameshift or point mutations.^{1–4} More than 90% of polyalanine expansion mutations have been considered to be *de novo*, and about 10% of the mutations were thought to have been inherited mostly from asymptomatic parents with somatic mosaicism and rarely from affected parents.^{4–6} Most recently, Bchetti *et al.*⁷ analyzed 52 parents using a sensitive method involving coupling amplification with carboxyfluorescein (FAM)-tagged primers and capillary electrophoresis, and found that 13 of the cases (25%) inherited the expanded mutations from a parent with late-onset central hypoventilation syndrome (LO-CCHS) or asymptomatic parents with somatic mosaicism or constitutive mutation. Herein, we studied the inheritance of expanded mutation in 45 unrelated families, and found that one patient (2%) inherited it from the mother with LO-CCHS, and nine patients (20%) inherited each mutation from apparently asymptomatic parent with somatic mosaicism.

MATERIALS AND METHODS

CCHS families analyzed

The ethics committee of the Yamagata University School of Medicine approved this study. After receiving written informed consent from the patients' families, peripheral blood was collected from family members for genomic DNA extraction. We studied 45 CCHS families with alanine expansion mutation. In all, 8 patients carried 5-alanine, 15 patients carried 6-alanine, 18 patients carried 7-alanine, 1 patient carried 11-alanine, 2 patients carried 12-alanine and 1 patient carried 13-alanine expansion mutations (Figures 1 and 2). One patient (case 79) was speculated to carry 7-alanine expansion mutation. This family had two deceased sibling patients complicated with Hirschsprung's disease, whose specimens were not available for genetic analysis. The parents cooperated with the study, and the father was identified as showing somatic mosaicism of 7-alanine expansion mutation (Table 1).

Molecular analysis of *PHOX2B*

We screened the coding region of *PHOX2B* by direct sequencing essentially according to the method described by Matera *et al.*⁸ For the analysis of somatic mosaicism of the family members of the patients carrying polyalanine expansion mutation, we amplified a 458-bp fragment or a 254-bp fragment including a polyaniline sequence in exon 3 from genomic DNA with primers 22F 5'-GCACTGACCCGGACAGCACT-3' and 3.3R 5'-TACCCGCTCGCCCA

¹Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan and ²Department of Neonatal Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan

Correspondence: Professor K Hayasaka, Department of Pediatrics, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan.
E-mail: hayasaka@med.id.yamagata-u.ac.jp

Received 11 December 2011; revised 22 February 2012; accepted 26 February 2012; published online 22 March 2012

Phenotypic variability in the family of case 17 carrying 5-alanine expansion mutation

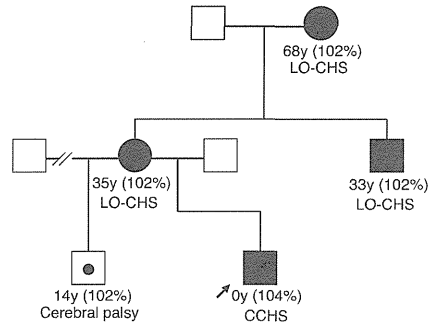


Figure 1 Phenotypic variability in the family of case 17 carrying 5-alanine expansion mutation. Filled squares and circles indicate affected males and females, respectively. Numbers under symbols and those in parentheses represent the age and percentage of mosaicism, respectively. The arrow indicates the proband. CCHS, congenital central hypoventilation syndrome; LO-CHS, late-onset central hypoventilation.

Number of alanine-expansion mutations in the studied patients and in the cases inherited from mosaic parents

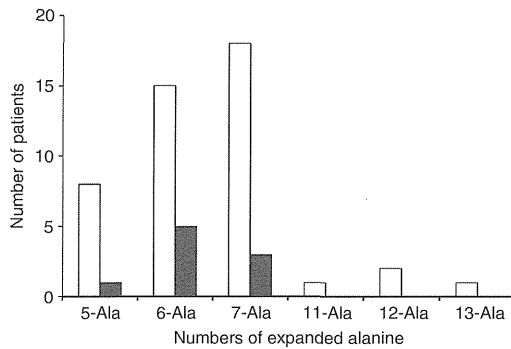


Figure 2 Number of alanine-expansion mutations in the studied patients and in the patients inherited from mosaic parents. Open and filled bars represent the studied patients and patients inherited from the mosaic parents, respectively.

CTCG-3' or with primers 22F 5'-GCACTGACCCCGACAGCACT-3' and 279R 5'-GAGCCAGCCTTGCCAGG-3'; the forward probes were 5' labeled with carboxyfluorescein (FAM) according to the method of Trochet *et al.*⁵⁹ and Bchetti *et al.*⁷ We loaded the PCR product on a 3130 Capillary Sequencer (PE Applied Biosystems, Foster City, CA, USA), used ROX500 (PE Applied Biosystems) as a size marker, and analyzed somatic mosaicism using GeneMapper version 4.1 software (PE Applied Biosystems). The percentage of mutant alleles was estimated by dividing the area under the peak corresponding to the mutant allele (E) by the sum of wild type (W) and mutant peak areas (E+W). Mosaicism of 5-, 6- and 7-alanine expansion mutations and mosaicism of 11-, 12- and 13-alanine expansion mutations were detected using primers with 22F and 3.3R, and using primers with 22F and 3.3R, respectively. To validate the technique and correct the percentage of mutant alleles, DNA solutions containing 1.25, 2.5, 3.75, 5, 12.5, 25, 32.5 and 50% of 5-, 6-, 7- and 13-alanine expansion mutations were prepared by mixing genomic DNA from controls and patients, of which the mixing ratio was

Table 1 Rate of mosaicism in the parents with somatic mosaicism

Case number	Sex	Parents (mosaicism)	Expanded alanine	Affected siblings	E/E+W (observed)	Mosaicism (%)
						(calculated)
Case 3	Female	Mother	6-Alanine	-	0.251	51
Case 41	Female	Father	6-Alanine	-	0.025	5
Case 46	Male	Father	6-Alanine	-	0.043	9
Case 55	Female	Father	6-Alanine	-	0.023	5
Case 68	Male	Mother	5-Alanine	+	0.306	62
Case 79	Male	Father	7-Alanine ^a	+	0.040	8
Case 84	Male	Mother	6-Alanine	+	0.269	55
Case 85	Male	Father	7-Alanine	-	0.028	6
Case 97	Female	Father	7-Alanine	-	0.280	58

The percentage of mutant alleles was estimated by dividing the area under the peak corresponding to the mutant allele (E) by the sum of wild type (W) and mutant peak areas (E+W).

^a7-alanine expansion was speculated from his father's data.

determined by the content of the template for the glyceraldehyde-3-phosphate dehydrogenase gene spanning from nucleotide 804 to nucleotide 903 (NM_002046.2) estimated by real-time PCR.¹⁰ Linear regression between the amounts of different alanine-expanded alleles and expected mosaicism levels were estimated with the degree of correlation measured by the R² coefficient. The equation of the regression line and R² coefficient for 5-, 6-, 7- and 13-alanine expansion mutation were $Y=0.49X$ (R²=0.9948), $Y=0.49X$ (R²=0.9998), $Y=0.48X$ (R²=0.9986) and $Y=0.46X$ (R²=0.9977), respectively.

To confirm the mosaicism of the parents detected by fragment analysis, a PCR fragment was subcloned into the TA cloning vector and the sequence of expanded clones was determined.

RESULTS

Among 45 CCHS families, we found that one patient inherited 5-alanine expansion mutation from a parent affected with LO-CHS (Figure 1), and nine patients inherited alanine expansion mutation from apparently asymptomatic parents with somatic mosaicism (Table 1). One patient inherited 5-alanine, five patients inherited 6-alanine, and three patients inherited 7-alanine expansion mutations from the parents with somatic mosaicism (Table 1, Figure 2). The percentage of mosaicism in each parent ranged from 5–62% and three patients had affected siblings. Six patients inherited expansion mutation from their fathers and three patients inherited it from their mothers.

Case 17, who was heterozygous for 5-alanine expansion mutation, presented with respiratory symptoms in the neonatal period, and his mother and grandmother were diagnosed with LO-CHS by polysomnography at the age of 35 and 68 years, respectively, following the diagnosis of the proband (Figure 1). His uncle had cardiac failure at the age of 31 years, and was confirmed to have LO-CHS by polysomnography. His half-brother, aged 14 years, was affected with cerebral palsy due to an unknown cause, but had no sleep apnea on polysomnography. The proband, half-brother, mother, uncle and grandmother were heterozygotes for 5-alanine expansion mutation (Figure 1).

DISCUSSION

Our study of 45 CCHS families revealed that one case (2%) inherited 5-alanine expansion mutation from the parent with LO-CHS (Figure 1) and nine cases (20%) inherited 5- to 7-alanine expansion mutation from the parents with somatic mosaicism (Table 1). Most CCHS patients carry 5- to 13-alanine expansion mutations in

PHOX2B, and more than 90% of its mutations had been considered to be *de novo*.⁴⁻⁶ However, Bachetti *et al.*⁷ analyzed 52 parents using a sensitive method, and found that 13 cases (25%) inherited the expanded mutations from their parents: 1 case inherited 5-alanine expansion mutation from a parent with LO-CHS, 2 cases inherited 5-alanine expansion mutation from asymptomatic parents with constitutive mutation, and 10 cases (five cases with 6-alanine, three cases with 7-alanine, 1 case with 9-alanine and 1 case with 13-alanine expansion mutation) from the parents with somatic mosaicism. As shown in Table 1, the level of mosaicism of each parent ranged from 5–62%. Three patients had affected siblings. The family of case 79 had two deceased sibling patients complicated with Hirschsprung's disease, whose specimens were not available for genetic analysis. Considering the recurrent mutation in this family, we analyzed the parents for genetic mosaicism, and detected the somatic mosaicism of 7-alanine expansion mutation in the father. The expanded mutation was definitely transmitted to the descendants even with the low level of mosaicism (5%) in the somatic genomes of the parent. The proportion of mutant alleles in somatic mosaicism may not reflect the proportion in germline mosaicism. Our study confirmed a high-frequency inheritance of alanine expansion mutation from mosaic parents and we would recommend testing all parents of CCHS proband with a sensitive fragment analysis.^{7,10}

The symptoms of the patients likely depend on the length of the expanded alanine. The individuals carrying 5-alanine expansion mutations are known to present central hypoventilation in the neonatal period or in a later period (LO-CHS), or are asymptomatic due to incomplete penetrance.¹¹ Therefore, the patients carrying 5-alanine expansion mutation would inherit the mutation from asymptomatic parents (parents with constitutive mutation or mosaicism) or parents affected with LO-CHS. Case 17 was a patient showing neonatal onset, and his mother and grandmother were diagnosed with LO-CHS by polysomnography at the age of 35 and 68 years, respectively, following the diagnosis of the proband (Figure 1). As for the symptoms in somatic mosaicism, nearly all individuals with somatic mosaicism were asymptomatic. However, one case mosaic for 8-alanine expansion mutation developed central hypoventilation in his forties.¹² The mosaic parents may require polysomnographic examination.

We previously reported that *de novo* alanine expansion mutation was of paternal origin and possibly caused by unequal sister chromatid exchange during spermatogenesis.¹³⁻¹⁵ However, a significantly high incidence of the mutation inherited from mosaic parents may raise the question of whether *de novo* polyalanine expansions are due to unequal crossover in the germline. No alanine-contracted-counterpart allele (expected counterpart allele in unequal crossover) was detected in individuals showing somatic mosaicism. In contrast, polymorphic alanine-contracted alleles were frequently detected in healthy individuals.^{1,3,4,16} It is also interesting to note that the most prevalent alanine-expanded allele is different between whole CCHS patients and the patients inherited from mosaic parents (Figure 2). Seven- and six-alanine-expanded alleles are most prevalent in whole CCHS patients and the patients inherited from mosaic parents, respectively (Figure 2).^{5-7,10} Therefore, alanine expansion mutation in somatic mosaicism is not likely caused by an unequal crossover, but by a replication mechanism such as a model of repeat instability generated during the replication fork stalling and restart within the repetitive run.¹⁷ Even though about 25% of the

alanine expansion mutations would be transmitted from the parents with mosaicism or constitutive mutation, a major part (about 75%) of mutation is likely *de novo*.

In conclusion, nearly 25% of CCHS patients carrying polyalanine expansion mutation inherit the mutation from parents with somatic mosaicism or constitutive mutation, and familial analysis using a sensitive method is needed for effective genetic counseling and the study of the alanine expansion mechanism.

ACKNOWLEDGEMENTS

We thank the patients and their families for their cooperation. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan.

- Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L. & Gener, B. *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. *Nat. Genet.* **33**, 459–461 (2003).
- Sasaki, A., Kanai, M., Kijima, K., Akaba, K., Hashimoto, M. & Hasegawa, H. *et al.* Molecular analysis of congenital central hypoventilation syndrome. *Hum. Genet.* **114**, 22–26 (2003).
- Matera, I., Bachetti, T., Puppo, F., Di Duca, M., Morandi, F. & Casiraghi, G. M. *et al.* *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset central hypoventilation syndrome. *J. Med. Genet.* **41**, 373–380 (2004).
- Weese-Mayer, D. E., Berry-Kravis, E. M., Zhou, L., Maher, B. S., Silvestri, J. M. & Curran, M. E. *et al.* 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. A* **123A**, 267–278 (2003).
- Trochet, D., O'Brien, L. M., Gozal, D., Trang, H., Nordenskjold, A. & Laudier, B. *et al.* *PHOX2B* genotype allows for prediction of tumor risk in congenital central hypoventilation syndrome. *Am. J. Hum. Genet.* **76**, 421–426 (2005).
- Berry-Kravis, E. M., Zhou, L., Rand, C. M. & Weese-Mayer, D. E. Congenital central hypoventilation syndrome: *PHOX2B* mutations and phenotype. *Am. J. Respir. Crit. Care Med.* **174**, 1139–1144 (2006).
- Bachetti, T., Parodi, S., Di Duca, M., Santamaria, G., Ravazzolo, R. & Ceccherini, I. Low amounts of *PHOX2B* expanded alleles in asymptomatic parents suggest unsuspected recurrence risk in congenital central hypoventilation syndrome. *J. Mol. Med. (Berl)*. **89**, 505–513 (2011).
- Matera, I., Bachetti, T., Puppo, F., Di Duca, M., Morandi, F. & Casiraghi, G. M. *et al.* *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset central hypoventilation syndrome. *J. Med. Genet.* **41**, 373–380 (2004).
- Trochet, D., Hong, S. J., Lim, J. K., Brunet, J. F., Munnich, A. & Kim, K. S. *et al.* Molecular consequences of *PHOX2B* missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. *Hum. Mol. Genet.* **14**, 3697–3708 (2005).
- Jennings, L. J., Yu, M., Zhou, L., Rand, C. M., Berry-Kravis, E. M. & Weese-Mayer, D. E. Comparison of *PHOX2B* testing methods in the diagnosis of congenital central hypoventilation syndrome and mosaic carriers. *Diagn. Mol. Pathol.* **19**, 224–231 (2010).
- Weese-Mayer, D. E., Berry-Kravis, E. M., Ceccherini, I., Keens, T. G., Dariu, A. & Lohmanee, D. A. *et al.* An official ATS clinical policy statement: Congenital central hypoventilation syndrome. Genetic basis, diagnosis, and management. *Am. J. Respir. Crit. Care Med.* **181**, 626–644 (2010).
- Trochet, D., de Pontual, L., Straus, C., Gozal, D., Trang, H. & Landrieu, P. *et al.* *PHOX2B* germline and somatic mutations in late-onset central hypoventilation syndrome. *Am. J. Respir. Crit. Care Med.* **177**, 906–911 (2008).
- Warren, S. T. Polyalanine expansion in synpolydactyly might result from unequal crossing-over of *HOXD13*. *Science* **275**, 408–409 (1997).
- Arai, H., Otogiri, T., Sasaki, A., Hashimoto, T., Umetsu, K. & Tokunaga, K. *et al.* *De novo* polyalanine expansion of *PHOX2B* in congenital central hypoventilation syndrome: unequal sister chromatid exchange during paternal gametogenesis. *J. Hum. Genet.* **52**, 921–925 (2007).
- Arai, H., Otogiri, T., Sasaki, A., Umetsu, K. & Hayasaka, K. Polyalanine expansion of *PHOX2B* in congenital central hypoventilation syndrome: rs17884724:A>C is associated with 7-alanine expansion. *J. Hum. Genet.* **55**, 4–7 (2009).
- Horiuchi, H., Sasaki, A., Osawa, M., Kijima, K., Ino, Y. & Matoba, R. *et al.* Sensitive detection of polyalanine expansions in *PHOX2B* by polymerase chain reaction using bisulfite-converted DNA. *J. Mol. Diagn.* **7**, 638–640 (2005).
- Mirkin, S. M. Expandable DNA repeats and human disease. *Nature* **447**, 932–940 (2007).



Original Article

Epidemiologic survey of patients with congenital central hypoventilation syndrome in Japan

Hisaya Hasegawa,¹ Kazuteru Kawasaki,² Hisashige Inoue,⁵ Minoru Umehara,³ Masato Takase⁴ and Japanese Society of Pediatric Pulmonology Working Group (JSPPWG)

¹Division of Neonatal Intensive Care, Tokyo Women's Medical University Medical Center East, ²Division of Pediatric Pulmonology, National Center for Child Health and Development, ³Umehara Children's Clinic, ⁴Department of Pediatrics, Nippon Medical School Tama Nagayama Hospital, Tokyo and ⁵Department of Pediatrics, Sumitomo Hospital, Osaka, Japan

Abstract *Background:* Congenital central hypoventilation syndrome (CCHS) is a rare disease characterized by hypoventilation during sleep. This study discusses the first epidemiologic survey of patients with CCHS in Japan.

Methods: The first survey was conducted between September and December 2006 and involved 507 registered institutes for pediatric training in Japan. The second survey was conducted between January and April 2007 and involved only those institutes that confirmed diagnosis of CCHS in the first survey or reported on CCHS at a conference during the preceding decade.

Results: Thirty-seven patients with CCHS were reported from 23 hospitals. Patient characteristics were as follows: 18 were male, 19 were female; and age range 4 months to 34 years. Diagnosis was based on clinical symptoms in 37/37 patients; blood gas analysis in 25/37; ventilatory response to inhaled CO₂ in 14/37; and genetic analysis (paired-like homeobox gene 2B) in 11/37. Complications included Hirschsprung's disease in 13/37 and central nervous system disorders in 15/37. Prognoses were as follows: 3/37 died in hospital, 1/37 remained in hospital, 33/37 were on home mechanical ventilation (died 4/33, survived 29/33), and 0/37 were cured. Ventilation methods included tracheostomy (21/37), use of a nasal mask (9/37), use of a facemask (5/37), and diaphragmatic pacing (1/37).

Conclusions: There is currently no consensus on the most appropriate methods for diagnosing and treating patients with CCHS in Japan. More CCHS-related data need to be collected in the near future in order to enable appropriate diagnosis and management of patients with CCHS.

Key words congenital central hypoventilation syndrome, epidemiologic survey, Japan.

Congenital central hypoventilation syndrome (CCHS) is a rare disease characterized by hypoventilation during sleep. It was first described by Mellins *et al.* in 1970.¹ The estimated incidence of CCHS is approximately 1 in 50 000 live births.² Recent evidence suggests that the clinical manifestations of CCHS correspond to the spectrum of clinical problems attributable to neural crest dysfunction.³ The paired-like homeobox gene 2B (*PHOX2B*) is the disease-defining gene for CCHS. Approximately 90% of individuals with the CCHS phenotype will be heterozygous for a polyalanine repeat expansion mutation. This study discusses the first epidemiologic survey of patients with CCHS in Japan.

Methods

The first survey of 507 registered institutes for pediatric resident training in Japan was conducted between September and

December 2006. The second survey was conducted between January and April 2007 and involved those institutes that confirmed diagnosis of CCHS in the first survey or reported on CCHS at a conference during the preceding decade.

Results

In the first survey, 35 of 507 institutes for pediatric training in Japan reported diagnosis of CCHS. A more detailed questionnaire for the second survey was delivered to these institutes, and 37 CCHS cases were identified from 23 institutes when duplicated data were excluded.

Patient background

There was no sex difference among the 37 cases (18 were male, 19 were female). The mean gestational age and mean birth body-weight were 39.2 ± 2.1 weeks and 2917 ± 360 g, respectively. Age ranged from 4 months to 34 years. Patients were widely distributed in terms of age, with small numbers in each age group, suggesting that a small number of cases develop each year

Correspondence: Hisaya Hasegawa, MD, PhD, Division of Neonatal Intensive Care, Tokyo Women's Medical University Medical Center East, 2-1-10 Nishiogu Arakawa-ku, Tokyo 116-8567, Japan. Email: hisaya@pst2.mfnet.ne.jp

Received 4 March 2010; revised 13 August 2011; accepted 13 September 2011.

© 2011 The Authors

Pediatrics International © 2011 Japan Pediatric Society

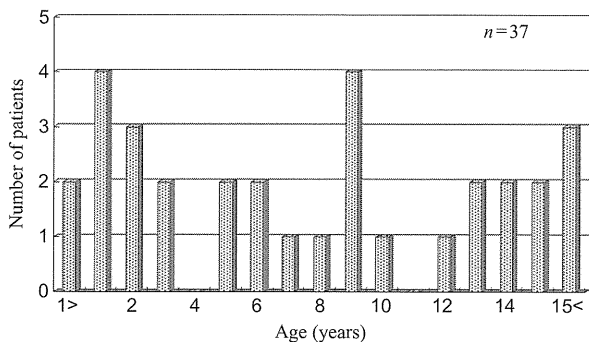


Fig. 1 Age distribution of patients with congenital central hypoventilation syndrome (CCHS).

(Fig. 1). Most cases occurred soon after birth. Onset at birth was common (29/37, 78%), whereas onset at an age greater than 1 year was rare (Fig. 2).

Diagnosis

Diagnoses were based on the following: clinical symptoms (37/37, 100%); blood gas analysis (25/37, 68%); ventilatory response to inhaled CO₂ (14/37, 35%); genetic analysis, namely *PHOX2B* analysis (13/37, 35%); pulmonary function test (9/37, 24%); and end-tidal CO₂ measurement (29/37, 24%) (Fig. 3). The time of diagnosis was late compared with the age of onset. Only four of the 37 patients (11%) were diagnosed within the first 6 days and 10 of the 37 (27%) within 1 month after birth (Fig. 4). Clinical symptoms of hypoventilation were observed when patients were awake (9/37, 24%) as well as asleep (37/37, 100%). *PHOX2B* defects were reported. *PHOX2B* is a regulator of the receptor tyrosine kinase (*RET*) gene. *RET* is related to differentiation and migration of neural crest cells. *PHOX2B* defects include polyalanine expansion and frameshift mutations. In this study, *PHOX2B* analysis was performed in 13/37 (35%) patients, and defects were observed in 11/13 (85%).

Background diseases and complications

Background diseases and complications were as follows: Hirschsprung’s disease (13/37, 35%), neurological complications,

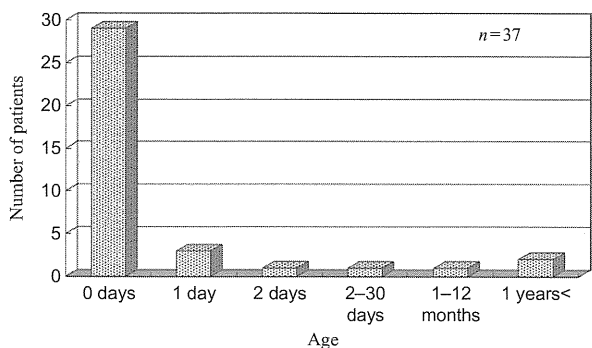


Fig. 2 Age at onset.

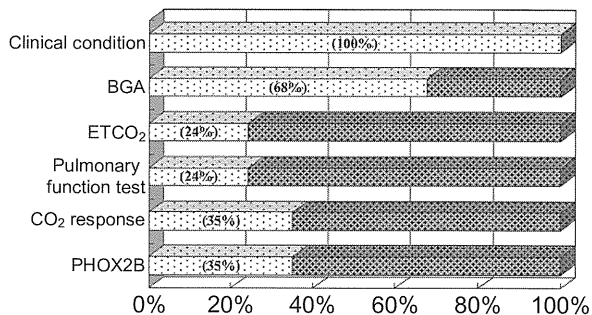


Fig. 3 Diagnosis-related examinations. BGA, blood gas analysis; CO₂ response, ventilatory response to inhaled CO₂; ETCO₂, end-tidal CO₂; PHOX2B, paired-like homeobox gene 2B.

such as epilepsy and psychomotor regression (15/37, 43%), airway diseases, such as bronchiectasis and acquired tracheal stenosis (2; 3/37, 8%), and pulmonary hypertension (2/37, 5%) (Fig. 5).

Ventilation methods

Ventilatory support approaches used in children with CCHS are summarized in Figure 6. Twenty-one patients received mechani-

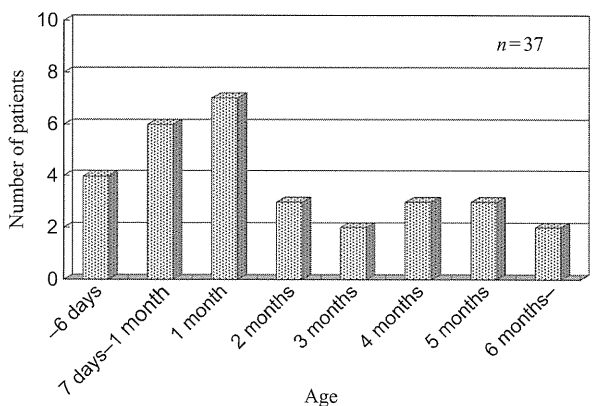


Fig. 4 Age at diagnosis.

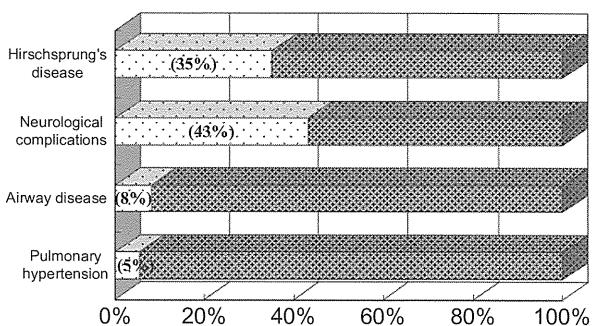


Fig. 5 Background diseases and complications.

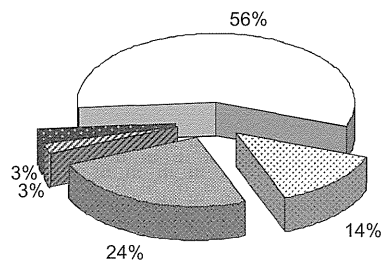


Fig. 6 Ventilation methods. □, ventilation via tracheotomy; ▨, facemask ventilation; ▩, nasal-mask ventilation; ▤, diaphragmatic pacers; ▥, ventilation via intratracheal tube.

cal ventilation via tracheotomy (21/37, 58%), and one patient received mechanical ventilation via an intratracheal tube (1/37, 3%). Fourteen patients received mask-based ventilation (a facemask in 5/37, 14%; a nasal mask in 9/37, 25%). Only one patient underwent diaphragmatic pacing (1/37, 3%). Non-invasive ventilation other than tracheotomy was performed in 15 of the 37 patients (40.5%). Twenty-eight patients received ventilatory support during sleep (28/37, 76%); nine patients required continuous ventilatory support (9/37, 24%) (Fig. 7).

Home mechanical ventilation and home monitoring

Home mechanical ventilation was administered to 33 of the 37 patients (89%). The youngest age at initiation of mechanical ventilation was 4 months, and the average age was 21 months. Most patients began home mechanical ventilation at 1 year (Fig. 8). Twenty-seven of the 33 patients (82%) used home monitoring with a pulse oximeter. With regard to home monitoring costs as reported by 22 patients, these were borne by the family (11/22, 50%), hospital (16/22, 28%), or supplier of the pulse oximeter (5/22, 23%).

Outcomes

Patients with CCHS survived without further disability (22/37, 59%) or with disability (8/37, 22%), or did not survive (7/22, 19%) (Fig. 9). Outcomes of the patients with CCHS included death in hospital (3/37, 8%), survival in hospital (1/37, 3%), and a shift to home mechanical ventilation (33/37, 89%). Among the 33 patients who received home mechanical ventilation, four died and 29 still required treatment. No patients recovered from CCHS.

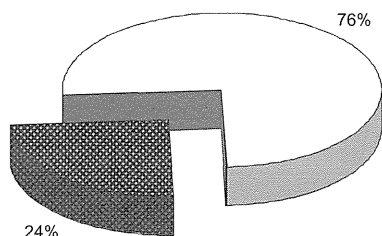


Fig. 7 Ventilatory support. □, sleep ventilation ($n = 28$); ▨, 24-h ventilation ($n = 9$).

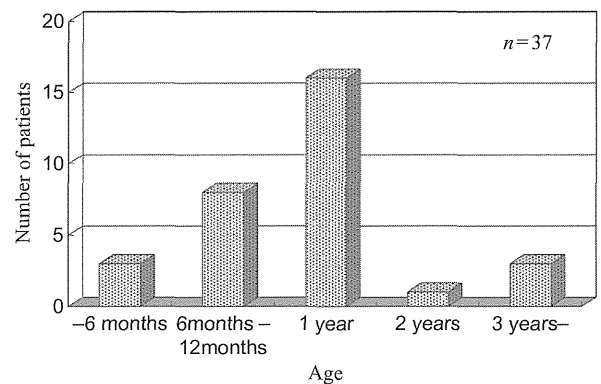


Fig. 8 Age at discharge.

Discussion

This is the first epidemiologic survey of CCHS in Japan. This study allows us to determine the actual state of CCHS in Japan. The methods for diagnosing and managing CCHS were varied and not integrated. Studies on CCHS have been reported abroad, including North America (94 cases)⁴ as well as the USA and Europe (196 cases).³ The present survey was conducted for pediatricians, whereas the foreign surveys were administered to Family Associations. Therefore, this study included evaluations of diagnostic methods that have not been reported in other studies.

Progress in diagnosis based on genetic testing should be noted for CCHS. In 2003, Amiel *et al.*⁵ reported on defects in *PHOX2B*, a regulator of *RET* that is related to differentiation and migration of neural crest cells. In Japan, Sasaki *et al.*⁶ examined 10 Japanese CCHS patients and reported that two of three had similar defects. Recently, testing methods have improved and have enabled detection of polyalanine expansion or frameshift mutations of *PHOX2B* in 92–100% of CCHS cases.^{7,8} It has thus been confirmed that defects of *PHOX2B* are the primary cause of CCHS. In this study, *PHOX2B* analyses were performed in 13/37 (35%) patients, and defects were observed in 11/13 (85%).

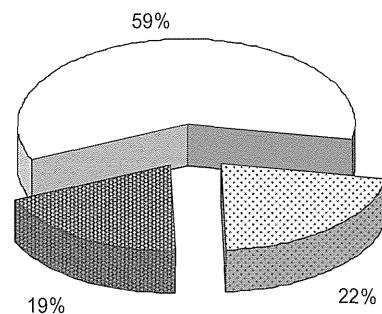


Fig. 9 Prognoses of patients with congenital central hypoventilation syndrome. □, survival without handicap; ▨, survival with handicap; ▥, death.

Hirschsprung's disease is the most well-known complication of CCHS. In this study, the rate of complication with Hirschsprung's disease was high (13/37, 35%). It has been reported that 16–50% of CCHS patients have Hirschsprung's disease.^{3,6,8–10} Recent studies have also suggested that *RET* may play a pivotal role as a modifier for the Hirschsprung's disease phenotype in patients with CCHS.^{11–14}

A high rate of neurological complications, such as epilepsy and psychomotor regression, were also observed (15/37, 43%). One study has reported that 30.7% of school-aged CCHS children have a learning disability, suggesting that the central nervous system might be affected by hypoxemia.¹⁵ This indicates that there are still some neurological problems in patients with CCHS, despite progress with regard to respiratory care. Monitoring via a pulse oximeter is indispensable for early detection of hypoxemia.

Non-invasive ventilation other than tracheotomy was performed in 15 of 37 patients (40.5%) in this study. This rate is almost equal to the average value in developed countries, including the USA (24.4%), France (35.3%), Italy (47.1%), the UK (66.7%), and Germany (66.7%).¹⁵ Home monitoring via a pulse oximeter was performed for 27 of the 33 patients (82%), which almost matches the rate reported in another study.¹⁵ The fact that no patient recovered from this disease suggests that patients with CCHS may require lifelong medical care. Therefore, it is necessary that the system enabling official support for adults be enhanced and that promotion of official support for the purchase of pulse oximeters is improved, as these are essential for home monitoring.

There was no consensus on the most appropriate method for the diagnosis of CCHS or of the most appropriate therapy for CCHS in Japan. Analysis of accumulated data on CCHS cases is required in the near future in order to ensure appropriate diagnosis and management of patients with CCHS.

References

- Mellins RB, Balfour HH Jr, Turino GM *et al.* Failure of autonomic control of ventilation (Ondine's curse). *Medicine (Baltimore)* 1970; **49**: 487–526.
- Gaultier C. *Congenital Central Hypoventilation Alveolar Syndrome: An Orphan Disorder. Lecture Abstracts, Second International Symposium on CCHS.* Faculte de Medecine Bichat, Universite Paris, Paris, 2002.
- Gozal D. Congenital central hypoventilation syndrome: an update. *Pediatr. Pulmonol.* 1998; **26**: 273–82.
- Vanderlaan MB. CCHS children in North America: medical and home-care issues. *Pediatr. Pulmonol.* 1997; **23**: 159–61.
- Amiel J, Laudier B, Attie-Bitach T *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat. Genet.* 2003; **33**: 459–61.
- Sasaki A, Kanai M, Kijima K *et al.* Molecular analysis of congenital central hypoventilation syndrome. *Hum. Genet.* 2003; **114**: 22–6.
- Weese-Mayer DE, Berry-Kravis EM, Zhou L *et al.* 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. A* 2003; **123**: 267–78.
- Weese-Mayer DE, Silvestri JM, Menzies LJ *et al.* Congenital central hypoventilation syndrome: diagnosis, management, and long-term outcome in thirty-two children. *J. Pediatr.* 1992; **120**: 381–7.
- Amiel J, Laudier B, Attie-Bitach T *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat. Genet.* 2003; **33**: 459–61.
- Trang H, Dehan M, Beaufilets F, Zaccaria I, Amiel J, Gaultier C, French CCHS Working Group. The French Congenital Central Hypoventilation Syndrome Registry: general data, phenotype, and genotype. *Chest* 2005; **127**: 72–9.
- Fitze G, Paditz E, Schlafke M *et al.* Association of germline mutations and polymorphisms of the *RET* proto-oncogene with idiopathic congenital central hypoventilation syndrome in 33 patients. *J. Med. Genet.* 2003; **40**: E10.
- de Pontual L, Pelet A, Trochet D *et al.* Mutations of the *RET* gene in isolated and syndromic Hirschsprung's disease in human disclose major and modifier alleles at a single locus. *J. Med. Genet.* 2006; **43**: 419–23.
- Fitze G, Konig IR, Paditz E *et al.* Compound effect of PHOX2B and *RET* gene variants in congenital central hypoventilation syndrome combined with Hirschsprung disease. *Am. J. Med. Genet. A* 2008; **146**: 1486–9.
- de Pontual L, Pelet A, Clement-Ziza M *et al.* Epistatic interactions with a common hypomorphic *RET* allele in syndromic Hirschsprung disease. *Hum. Mutat.* 2007; **28**: 790–6.
- Vanderlaan M, Holbrook CR, Wang M *et al.* Epidemiologic survey of 196 patients with congenital central hypoventilation syndrome. *Pediatr. Pulmonol.* 2004; **37**: 217–29.

特集

最近 10 年で最も進歩した研究分野を検証する

新生児の呼吸器治療学の進歩

長谷川 久弥

長谷川 久弥：新生児の呼吸器治療学の進歩。呼吸 31(9)：868-874, 2012
 キーワード：新生児、人工呼吸、呼吸機能検査、気管支鏡検査

新生児領域における呼吸器疾患の管理、治療の進歩は目覚ましく、重症呼吸器疾患をもつ新生児に大きな福音をもたらしている。本稿では、ここ 10 年で大きく進歩した新生児領域特有の呼吸管理、ベッドサイドで施行可能な呼吸機能検査、気管支鏡検査などを中心に述べる。

I. 新生児用人工呼吸療法¹⁾

人工呼吸療法の進歩は新生児医療の分野でも大きな効果をもたらしている。高頻度振動換気(high frequency oscillation: HFO)など、成人領域に先がけて発展してきた人工換気療法もある。一般の人工換気療法でいえば、成人領域では患者の自発呼吸に合わせて人工呼吸を行う patient triggered ventilation (PTV) が早くから取り入れられているのに対し、新生児領域では、長い間、間欠的強制換気(intermittent mandatory ventilation: IMV)が用いられてきた。1990 年代に入り、新生児領域においても PTV の概念が導入されるようになり、新しい人工呼吸器が開発されてきている。また、持続陽圧呼吸法(continuous positive airway pressure: CPAP)も新しい方式を加え、再び有用性が見直されてきている。

1. 持続陽圧呼吸法 (continuous positive airway pressure: CPAP)

呼吸障害のある児では呻吟がみられることがある。これ

は児が呼気時に声帯を狭め、肺胞が虚脱するのを防ぎ、機能的残気量を増やそうとしている自己防衛反応である。このことを臨床的に応用し、持続的に陽圧を負荷する方法が CPAP である。CPAP を行うことにより、① 動脈血酸素化の改善、② 機能的残気量の増加、③ 無呼吸発作の減少、④ 気道保持、などが期待できる。しかし、CO₂ を低下させる効果は期待できないため、CO₂ が上昇するような病態では他の換気法の選択が必要となる。CPAP の施行法としては、鼻呼吸を利用した nasal prong による nasal CPAP と気管挿管による endotracheal CPAP とがある。nasal CPAP は 1970 年代には新生児呼吸障害の管理法として広く普及していたが、1980 年代には新生児用人工呼吸器の普及とともに気管挿管による人工呼吸管理にとって代わられていた。1987 年に北米 8 カ所の NICU の比較検討で、nasal CPAP を積極利用していた Colombia 大学の慢性肺疾患 (CLD) の発症頻度が低いことが注目され、nasal CPAP の再評価につながった²⁾。nasal CPAP は気管挿管を回避できることから、気管挿管に伴う気道感染や喉頭浮腫、声門狭窄などの合併症予防の意味からも利点が見出せる。また、新生児の医療現場においては既に主要な呼吸管理装置の位置を取り戻しており、様々な新製品が開発されている。nasal CPAP は、その方式の違いから大きく 2 つに分類される。1 つは圧を気道にかけることで保持をはかる従来の CPAP であり、もう 1 つは flow で気道の保持をはかる DPAP (directional positive airway pressure) である (図 1)。前者の代表が通称川口式と呼ばれるアトムメディカル社製 nasal prong で、通常の人工呼吸器で使用可

The development of respirology in neonates
 東京女子医科大学東医療センター 新生児科
 Hisaya Hasegawa
 Division of Neonatal Intensive Care, Tokyo Women's Medical
 University, Medical Center East, Tokyo 116-8567, Japan

能、フィット性がよく鼻中隔への影響が少ない、腹臥位使用が可能で抑制も必要としないなどの特徴をもち、簡便な nasal CPAP 装置として臨床の場で広く用いられている。後者の代表は Infant flow driver systemTMによる nasal DPAP 装置で、コアンダ効果により吸気だけでなく、呼気の呼吸仕事量も軽減させる特徴をもっている(図2)。専用の装置を必要とし、腹臥位での使用が困難、鼻中隔の保護が必要など、簡便性では劣るが、呼吸仕事量を軽減させるため、呼吸筋の弱い超低出生体重児・極低出生体重児において頻用される。

CPAP 圧は 5~7 cmH₂O 程度で行うが、気管・気管支軟化症など気道の閉塞を来しやすい疾患では、より高い 7~10 cmH₂O 程度の圧で管理する。

2. 高頻度振動換気 (high frequency oscillation : HFO)

通常の人工換気法とは異なり、生理的な換気回数を著しく超えた換気回数、少ない 1 回換気量で人工換気を行う換気法が HFO である。通常の換気では 1 回換気量が死腔量より少ない状態では CO₂ の蓄積が起こってしまう。しかし、換気回数を極端に増加させていくと死腔量以下の 1 回換気量でも isocapnea を保つことができるようになる³⁾。ガス交換の機序としては通常の換気療法とは異なり、拡散を中心としたガス交換が主体と考えられている。HFO の特徴は① 1 回換気量が死腔量より少ない、② 換気回数が 3Hz 以上、③ active expiration が可能、な点である。肺を膨らませた状態で、少ない 1 回換気量で振動させて換気を行うため、換気条件の厳しい児や超低出生体重児などで、より肺損傷の少ない人工換気が可能である(図3)。HFO は気道に閉塞性病変の伴わない air block 症候群、呼吸窮迫症候群などで有効であるが、閉塞性病変を伴うものには無効である。

HFO の方式には、① ピストン方式、② スピーカー方式、③ 気流遮断方式、④ ジェット方式などがある。HFO では、PaCO₂ は 1 回換気量(振幅度)と換気回数で調節するが、換気回数は通常固定して用いるため 1 回換気量(振幅度)で調節する。PaO₂ は吸入酸素濃度と平均気道内圧(MAP)で調節する。HFO においてはこれらのパラメータを独立してコントロールすることが可能である。

HFO で呼吸管理を行ううえで重要なことは、十分な MAP をかけた状態で HFO を行うことである (high MAP strategy)⁴⁾。低い MAP で HFO を行うと振動が肺泡でなく末梢気道に直接作用し、気道損傷の原因となる。5 cmH₂O では HFO の効果はなく、6 cmH₂O で間質性肺気腫が多発することから、最低でも 7 cmH₂O の MAP は保つようにする。

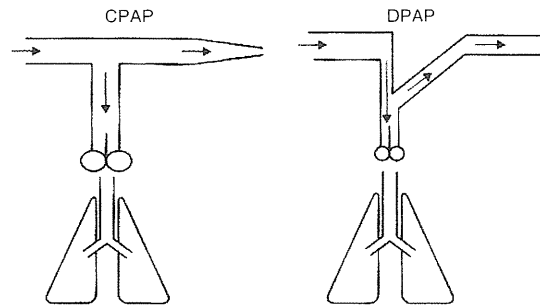


図1 CPAP と DPAP

II. ベッドサイドでの呼吸機能検査⁵⁾⁶⁾

1. 肺機能検査

呼吸機能検査は肺の状態を直接評価する有力な検査である。新生児領域の呼吸機能検査の他領域と最も異なる点は、児の協力が得られない点である。このため、新生児の呼吸機能検査は児の協力が得られなくても臨床に役立つ結果が得られるように様々な工夫が行われてきた。換気量などの測定方法として代表的なものは body plethysmography と pneumotachography である。body plethysmography は児の体を密閉したチャンバー内に収容し、肺容量の変化をチャンバー内の圧変化として測定し、Boyle の法則を用いて換気量を求める方法である。換気量の測定以外に肺容量の測定も可能で、研究を行ううえでは有用な方法であるが、装置は大きく高価であり、状態の悪い児では測定条件を満たすことが困難であることなどから、現在のところ臨床応用はあまりなされていない。

臨床の場で主に用いられているのは pneumotachography である。pneumotachography は気流が層流であると流速と圧差の間には比例関係がみられることを利用し、層流ができるように作られた抵抗を用い、その前後の圧差を測定することにより流速を求め、流速を積分することにより換気量を求める方法である。

現在市販されている呼吸機能測定装置は Fleisch 型 pneumotachograph とパソコンを組み合わせ、換気量だけでなく、compressd air で開閉するバルブを用いた passive flow-volume technique⁷⁾などにより、様々な呼吸機能の測定が可能となっている。現在、本邦で最も普及していると思われるアイビジョン社製呼吸機能測定装置(図4)を用いた測定法を例に述べる。この装置はベッドサイドに簡単に移動でき、pneumotachograph を選択することにより超未熟児から成熟児まで測定可能である。マス

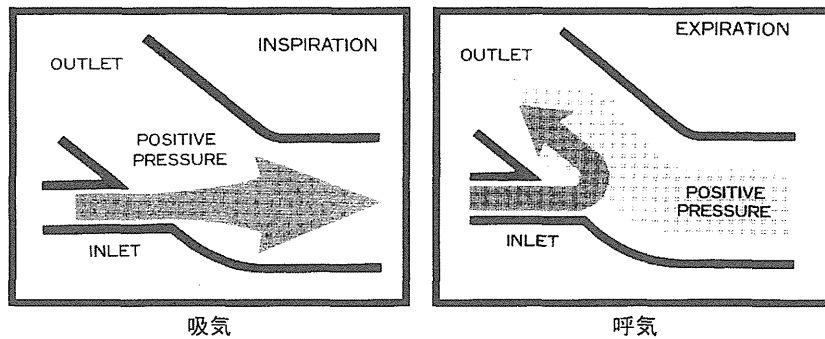


図2 Nasal DPAP によるコアンダ効果

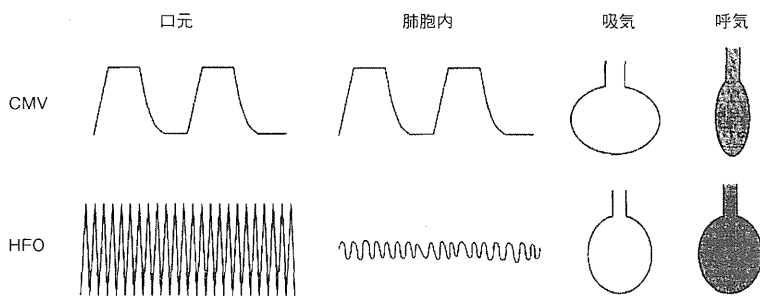


図3 CMV と HFO

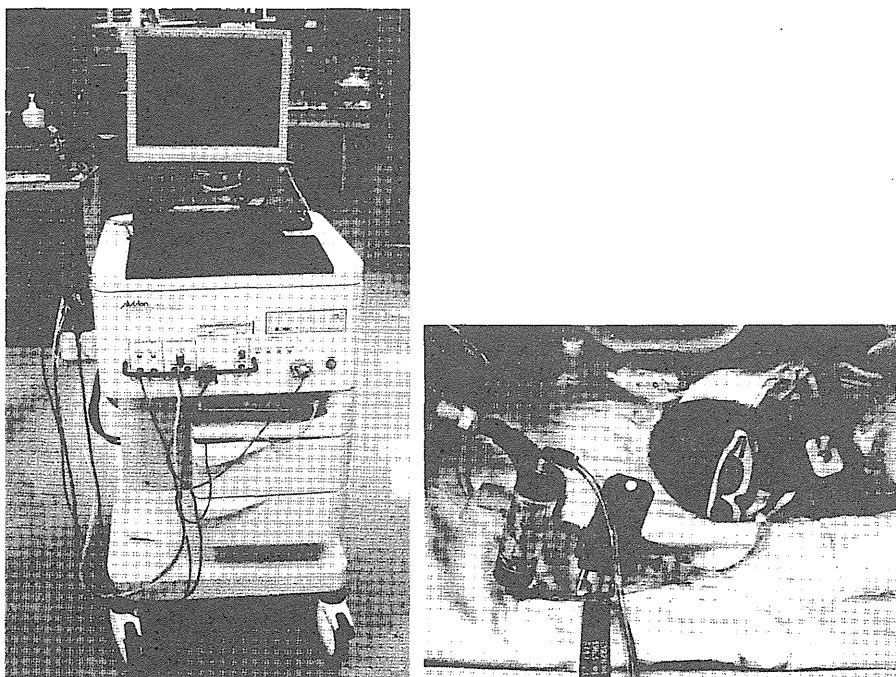


図4 アイビジョン社製呼吸機能測定装置

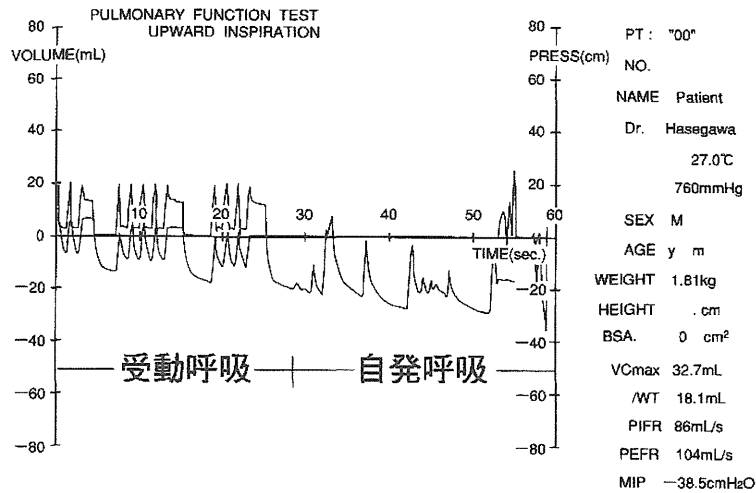


図5 呼吸機能測定画面
上側が圧曲線、下側が換気量曲線。前半は受動呼吸、後半は自発呼吸による測定。

表1 新生児呼吸器疾患の疾患別肺機能の特徴

	RDS	TTN	MAS	Pn.	BPD	WMS
Crs	↓	↓	↓	↔	↓	↔
Rrs	↓	↓	↑	↑	↑	↔
CVC	↓	↔	↓	↔	↓	↔

RDS: respiratory distress syndrome. TTN: transient tachypnea of the newborn. MAS: meconium aspiration syndrome. Pn.: pneumonia. BPD: bronchopulmonary dysplasia. WMS: Wilson-Mikity syndrome

ク法による測定も可能であるが、原則として気管挿管下の児が対象となる。測定は passive flow-volume technique を用いた前半の受動呼吸部分と自発呼吸を出させた後半部分とからなっており、全部で1分間で終了する。前半の受動呼吸部分を解析することにより、静肺胸郭コンプライアンス(Crs)、気道抵抗(Rrs)、時定数(TC)等が求められ、後半の自発呼吸部分で啼泣時肺活量(CVC)などを求める(図5)。

1) 臨床応用

ベッドサイドで測定した呼吸機能は、その場で診断、治療に役立たせることができる。

(1) 抜管基準

呼吸機能を測定することにより、確率の高い抜管が可能となる。抜管基準を以下に示す。

成熟児: ① Crs 0.6 ml/cmH₂O/kg 以上, ② CVC 15 ml/kg 以上

未熟児: 無呼吸の心配のある未熟児では上記①、②の基準に加え、気道閉塞法による中枢性呼吸機能の評価も行い、

③ %prolongation + 10 %以上

(2) 診断

新生児の呼吸器疾患は疾患ごとに特徴的な呼吸機能を呈する。胸部 X 線などと組み合わせることにより、よりの確な診断が可能となる。各疾患の呼吸機能の特徴を表1に示す。

(3) その他

人工肺サーファクタントをはじめとする薬剤の適応の決定、治療効果の判定など様々なことに応用可能である。

2. 呼吸耐力検査 (breathing intolerance index: BITI)

BITI は呼吸筋の呼吸耐力を反映する指標として用いられている。呼吸筋の疲労は、① 全呼吸時間(T_{tot})に対する吸気時間(T_i)の割合(T_i/T_{tot})が増加するほど速やかに疲労し、② 毎回の呼吸筋の収縮力(1回換気量: TVで代用)の最大収縮力(努力性肺活量: VCで代用)に対する割合が増加するほど速やかに疲労することが報告されている。BITIはこの2つの要素を掛け合わせた BITI=(T_i/

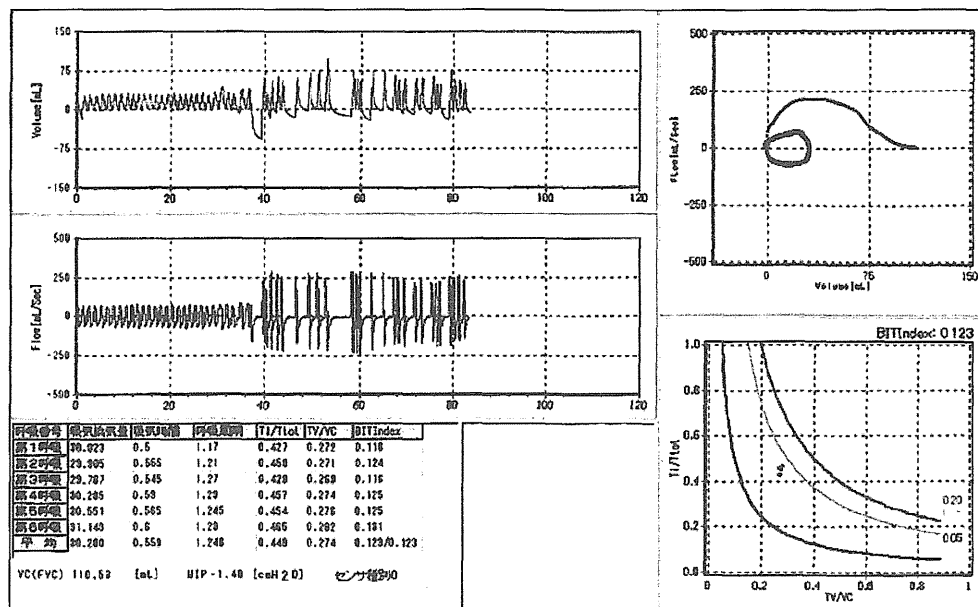


図6 BITIの測定・解析画面

$T_{tot} \times (TV/VC)$ の式で求められる(図6)。BITIが0.15以上の症例では換気補助を必要とするが、成人に比し、新生児ではより余力が少ない状態となっている。

3. 中枢性呼吸機能検査

呼吸調節は複雑なメカニズムで行われているが、大きく分けると伸展受容器などを介した反射性呼吸調節と CO_2 に対する化学性呼吸調節に分類される。反射性呼吸調節を検査する方法としては気道閉塞法があり、化学性呼吸調節を検査する方法としては CO_2 responseがある。

1) 気道閉塞法

気道閉塞法は安静時の呼吸に比べ気道閉塞時の呼吸がどのように変化するかをみることにより反射性呼吸調節を調べる方法である。測定は安静呼吸が数呼吸続いたことを確認した後、気道閉塞直前の1呼吸をコントロールの呼吸としてコンピューターに認識させ、呼気末で自動的に弁を閉鎖し気道を閉塞させる。吸気努力が続いている間は弁を閉じ続け、吸気努力の終了を感知すると自動的に弁を開放し安静呼吸に戻る。気道閉塞時の陰圧波形を解析することにより、気道閉塞後0.1秒の陰圧(inspiratory pressure 100 msec. after airway occlusion: P_{100})、最大吸気圧(maximum inspiratory pressure: MIP)、MIPに対するコントロールの呼吸の換気量の比(effective elastance: E_r)、コントロールの呼吸の吸気時間に対する気道閉塞時の吸気努力の持続時間の延長度(%prolongation)などを測定する(図7)。%prolongation + 10%以上で無呼吸発作の減少

がみられる。

2) CO_2 喚起応答試験

CO_2 に対する換気応答を調べる検査で、濃度を変えた CO_2 下で呼吸をさせ、それぞれの CO_2 濃度における換気応答を調べる steady-state 法と再呼吸回路を用い連続的に CO_2 を蓄積させ換気応答を調べる再呼吸法とがある。検査時間や児にかかる負担などから、最近では再呼吸法が行われる場合が多くなっている。再呼吸法は5% CO_2 +95% O_2 の混合ガスを閉鎖回路内で呼吸させ、 CO_2 濃度が約10%になるまで再呼吸を続け CO_2 濃度と換気量の関係をコンピューターで解析する(図8)。中枢性肺泡低換気症候群(CCHS)の例では、 CO_2 の蓄積に対する換気応答は著しく低下する。

III. 気管支鏡検査^{B)}

新生児領域において呼吸器疾患の占める割合は大きく、また呼吸管理の進歩などにより、治療対象となる疾患も多様化してきている。こうしたなかで、直接的に気道を観察できる気管支鏡は診断、治療の有力な選択肢となっている。

新生児領域における気管支鏡検査の適応としては大きく2つがある。1つは胸部X線での異常陰影、喘鳴、陥没呼吸などの呼吸障害を呈する児で、気道病変が凝われる際の診断確定のために行われる場合。もう1つは気管挿管、気管切開などを施行されている児で気道病変の予防、早期発

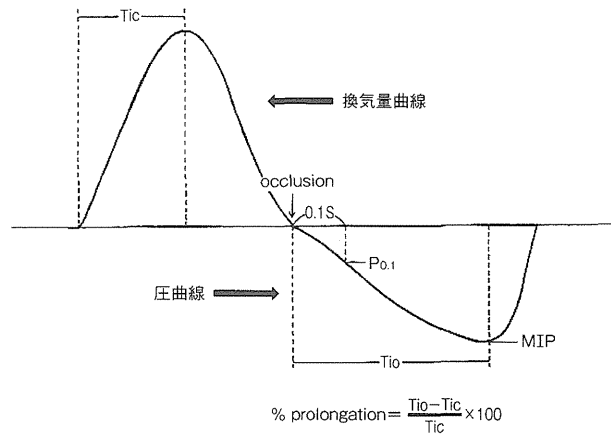


図7 反射性中枢性呼吸機能検査(気道閉塞法)

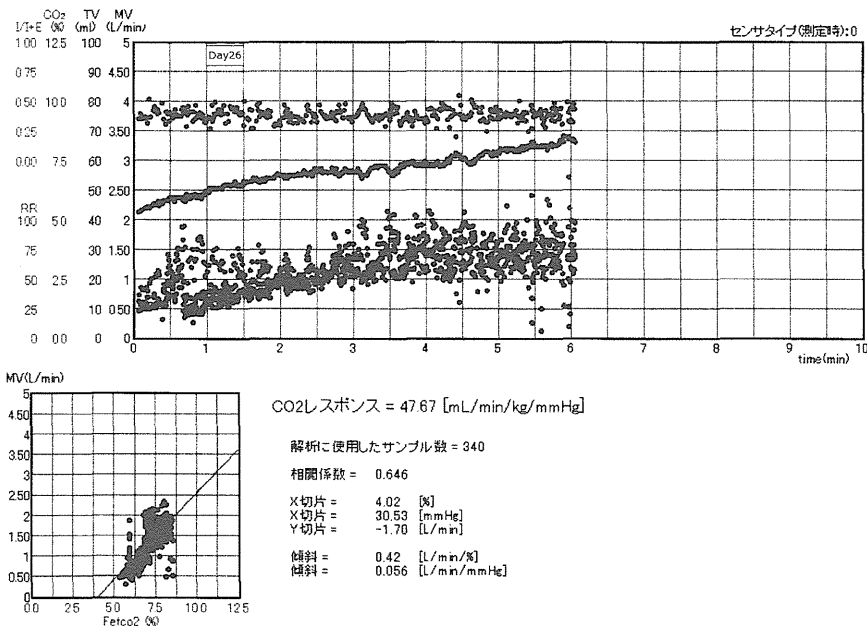


図8 化学性中枢性呼吸機能検査(CO₂ response)の測定・解析画面

見のためのスクリーニング検査として行われる場合である。スクリーニング検査は必ずしも全例で行う必要はないが、早期に病変に気付いて対応することで、重篤な気道病変を回避することが可能となる。

新生児領域で用いる気管・気管支鏡は軟性の気管支ファイバースコープで、観察用としては外径1.4~2.4mmの処置チャンネルのないタイプのものを使用し、処置用としては外径2.3~3.0mmの処置チャンネルの付いたタイプのものを使用する(図9)。処置チャンネルを使用すること

により、気管肉芽レーザー焼灼術、難治性無気肺の治療なども可能である。また、細径の鉗子等も開発され、気道病変の治療に用いられている(図10)。

IV. 一酸化窒素(NO)吸入療法

一酸化窒素(NO)吸入療法は、1992年にRoberts⁹⁾、Kinsellaら¹⁰⁾が新生児遷延性肺高血圧症(PPHN)に対する効果を報告して以来、画期的な治療法として注目されて

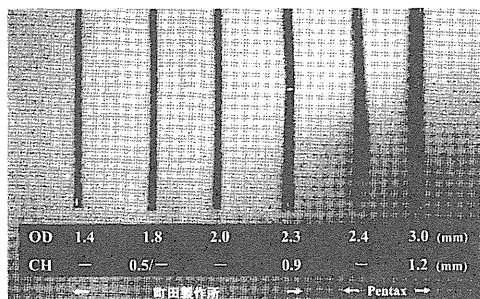


図9 新生児・小児用気管支ファイバースコープ

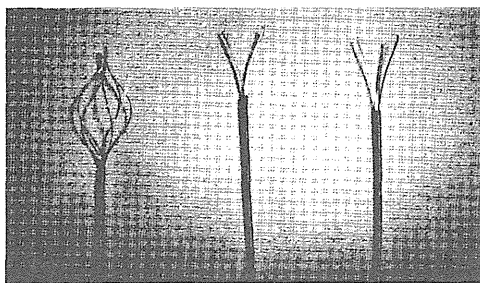


図10 新生児・小児気道処置用鉗子
バスケット鉗子(左)、2本爪鉗子(中)、3本爪鉗子(右)

きた。NO 吸入療法が優れている点は、選択的に肺動脈圧を低下させるため、体血圧に影響を与えないことである。このことから、PPHN に対してだけでなく、先天性心疾患の術後肺高血圧症、低心拍出量症候群などに対しても、有用性が認知されている。米国では1999年、欧州では2001年にNO吸入療法の承認が得られて臨床使用され、日本では2008年に薬事承認がなされて使用されている。

現在、使用されているNO吸入療法装置は、専用のNOガス(INOflow)およびNO供給システム(INOvent)である(図11)。呼吸回路にNOを供給しながら、NO、NO₂をモニタリングするシステムで、一体化されているため準備・施行が容易にできるようになっている。NO吸入療法は生後7日以内に吸入濃度20ppmで開始。開始後4時間は20ppmを維持し、酸素化の改善に伴い5ppmに減量する。投与中止は、吸入濃度を1ppmまで徐々に減量し、問題なければ、FiO₂を0.1増量し、NO吸入を中止する。高濃度NOを供給した場合、メトヘモグロビン血症を来すことがあるため、メトヘモグロビンの測定を行い、2%を超えないように管理する。

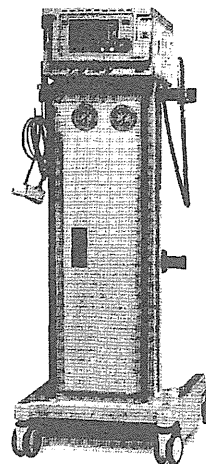


図11 INOvent delivery system

おわりに

新生児の呼吸管理はこの10年間で大きく進歩した。最近では新生児期の肺障害が将来的な肺機能に大きな影響を及ぼすことも分ってきている。長期的な肺機能予後改善のために、新生児科、小児科、呼吸器内科などが連携していくことが望まれる。

文 献

- 1) 長谷川久弥, 新生児の人工呼吸療法. 小児科診療 67:2167-2175, 2004
- 2) Avery ME, Tooty WH, Keller JB, *et al.* Is chronic lung disease in low birth weight infants preventable? A survey of eight centers. *Pediatrics* 79:26-30, 1987
- 3) Bryan AC. High frequency oscillation. *周産期医学* 15:1343-1349, 1985
- 4) 堺 武男, 高頻度振動換気法. 小児内科 29:435-442, 1997
- 5) 長谷川久弥, 新生児の呼吸機能. 未熟児新生児誌 5:41-58, 1993
- 6) 長谷川久弥, 新生児呼吸機能検査の臨床応用. 東京女子医科大学学会雑誌 81:165-170, 2011
- 7) LeSouef PN, Lopes JM, England SJ, *et al.* Passive respiratory mechanics in newborns and children. *Am Rev Respir Dis* 129:552-556, 1984
- 8) 長谷川久弥, 新生児気道病変の管理. 日本未熟児新生児学会雑誌 18(1):29-37, 2006
- 9) Roberts JD, Polaner DM, Lang P, Zapol WM. Inhaled nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 340:818-819, 1992
- 10) Kinsella JP, Neish SR, Shaffer E, Abman SH. Low-dose inhalation nitric oxide in persistent pulmonary hypertension of the newborn. *Lancet* 340:819-820, 1992



- 6 Kaulfersch W, Urban C, Hauer C *et al.* Successful treatment of CMV retinitis with ganciclovir after allogeneic marrow transplantation. *Bone Marrow Transplant.* 1989; **4**: 587–9.
- 7 Miyoshi H, Tanaka-Taya K, Nagae Y *et al.* Cytomegalovirus retinitis after transplantation of positively selected CD34+ cells from HLA-mismatched donors. *Pediatr. Infect. Dis. J.* 1998; **17**: 345–8.
- 8 Bauml CR, Levin AV, Read SE. Cytomegalovirus retinitis in immunosuppressed children. *Am. J. Ophthalmol.* 1999; **127**: 550–8.
- 9 Song WK, Min YH, Kim YR, Lee SC. Cytomegalovirus retinitis after hematopoietic stem cell transplantation with alemtuzumab. *Ophthalmology* 2008; **115**: 1766–70.
- 10 El-Chennawi FA, Al-Tonbary YA, Mossad YM, Ahmed MA. Immune reconstitution during maintenance therapy in children with acute lymphoblastic leukemia, relation to co-existing infection. *Hematology* 2008; **13**: 203–9.

Slowly progressive sleep apnea in late-onset central hypoventilation syndrome

Takehisa Fujiwaki,^{1,2} Hisaya Hasegawa,³ Hiroko Arai,⁴ Kiyoshi Hayasaka⁴ and Shigeru Ohta²

¹Department of Pediatrics, Ohchi Hospital, Ohnan-cho, ²Department of Pediatrics, Matsue Red Cross Hospital, Matsue, Shimane, ³Division of Neonatal Intensive Care, Tokyo Women's Medical University Medical Center East, Tokyo and ⁴Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan

Key words late-onset central hypoventilation syndrome, Ondine's curse, *PHOX2B* gene.

Congenital central hypoventilation syndrome (CCHS or Ondine's curse) is a life-threatening disorder involving impaired ventilatory responses to hypercapnia and hypoxemia resulting in sleep hypoventilation. The paired-like homeobox 2B (*PHOX2B*) gene was identified as the causative gene of CCHS. Patients with CCHS generally suffer from hypoventilation, which becomes more pronounced during sleep, immediately after birth or at least within a few days. Recently, some patients were reported to experience sleep hypoventilation later in life, so-called late-onset central hypoventilation syndrome (LO-CHS). In this paper, we present a case with LO-CHS with an atypical clinical course, that is, the severity of the sleep hypoventilation gradually deteriorated over several years, and additional symptoms that might have been possible bad effects of the oxygen supply appeared after the start of home oxygen therapy.

Case Report

The patient was a Japanese boy born to healthy parents without consanguinity: a 40-year-old father and a 38-year-old mother. No relatives of either parent suffered from sleep disturbance. A nine-year-old elder sister, who is healthy and has no sleep problems, is the only sibling. The patient was delivered naturally transvaginally as a full-term infant without any complication during pregnancy and delivery. In the newborn period he required phototherapy for neonatal hyperbilirubinemia with favorable

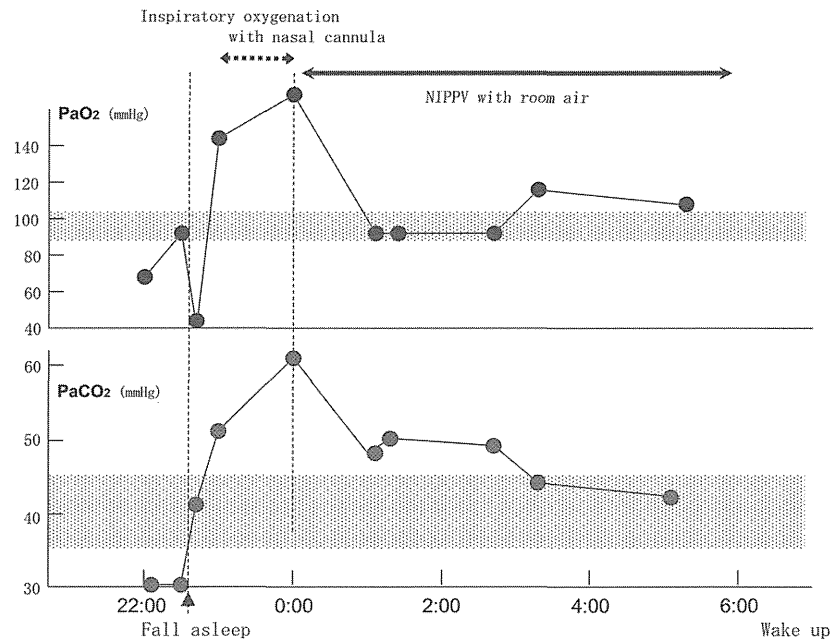
improvement, other symptoms not being noticed. After good growth and development, at 1 year and 2 months of age he was hospitalized due to respiratory failure caused by severe pneumonia, when hypoventilation was noticed in sleep for the first time. During sleep he exhibited reduced respiratory thorax movement, hypoxia and hypercapnia, all of which quickly deteriorated after he fell asleep but improved when he woke up. He required assist-control ventilation. He also suffered from hypothyroidism and auxocardia, and was thus given denopamine, furosemide, spironolactone and levothyroxine sodium hydrate. As the pneumonia improved, the sleep hypoventilation disappeared; he was discharged from the hospital and lived a healthy life at home. He was noticed to exhibit developmental delay at 1 year and 6 months of age. At 2 years and 8 months of age he underwent cardiac catheter examination to determine the cause of auxocardia, edema, and appetite loss, which were symptoms/signs first noticed at 1 year and 2 months of age and then persisted; he was diagnosed as having secondary pulmonary hypertension caused by sleep hypoventilation without any heart anomaly. Although between 1 year and 2 months of age and 3 years of age nocturnal sleep apnea was not obvious, he could not sleep soundly and tended to easily wake up. After 3 years of age, sleep hypoventilation episodes occasionally occurred associated with respiratory infection, so he started to undergo home oxygen therapy during nocturnal sleep. After the start of home oxygen therapy, SpO₂ during nocturnal sleep improved, while the frequency of sleep hypoventilation accompanied by respiratory infection increased. Each time he suffered from sleep hypoventilation at home, the parents, who heard the patient's SpO₂ monitor alarm, woke him up by tapping and shaking. At that time, he began to suffer from occasional paroxysmal episodes of severe anxiety and excitement

Correspondence: Takehisa Fujiwaki, MD, Department of Pediatrics, Ohchi Hospital, 3848-2 Nakano, Ohnan-cho, Shimane 696-0193, Japan. Email: take-fujiwaki@k7.dion.ne.jp

Received 13 November 2009; revised 18 March 2011; accepted 13 June 2011.

doi: 10.1111/j.1442-200X.2011.03431.x

Fig. 1 Blood gas analysis during night sleep with inspiratory oxygenation and nasal intermittent positive pressure ventilation (NIPPV) in a hospital at 5 years and 10 months of age. The upper and lower panels show the time courses of PaO₂ and PaCO₂, respectively. As soon as the patient fell asleep, PaO₂ decreased and PaCO₂ increased immediately. Following inspiratory oxygenation with a nasal cannula, PaO₂ increased in response to it, while PaCO₂ continued to increase further. Subsequent NIPPV with room air improved both PaO₂ and PaCO₂ successfully until he woke up. The shadowed areas indicate the normal ranges for PaO₂ and PaCO₂, respectively.



for approximately half an hour in the daytime. As time passed the frequency and severity of the nocturnal sleep hypoventilation gradually increased. At 4 years and 4 months of age he suffered from the first seizure during night sleep. At 5 years and 9 months of age, amino acid analysis, organic acid analysis, head computed tomography, head magnetic resonance imaging, electroencephalography, auditory brainstem response audiometry, somatosensory evoked potential examination, and visually evoked potential examination were performed for the patient, all being normal. Within 2 months from 5 years and 9 months of age, he suffered from seizures four times during nocturnal sleep under home oxygen therapy. Simultaneously, his sleep apnea began to occur more frequently, and it became so difficult for him to recover from it with stimulation and/or assisted ventilation using a bag and mask that he needed controlled ventilation with tracheal intubation during sleep every night. He had to undergo tracheal intubation on falling asleep and extubation on waking up on consecutive days, subsequently suffering from hoarseness, and thus was judged to be at the limit of daily intubation/extubation. Therefore, he was admitted to Matsue Red Cross Hospital at 5 years and 10 months of age.

The patient could understand our directions well and act on them, even though he could not utter any meaningful words. He did not suffer from dyspnea. He had no clinical features of neuromuscular, cardiac or respiratory disease. Blood examination revealed slight lowering of the thyroid hormone level. No airway stenosis or anomaly was observed in his pharynx or larynx on laryngeal fiberoptic examination. However, at night his respiratory chest movement remarkably decreased immediately after he fell asleep, and his breath sound became inaudible. He underwent nasal intermittent positive pressure ventilation (NIPPV) with a pressure-controlled respirator (Fig. 1). After the

tenth day of hospitalization he was successfully treated with NIPPV using a portable ventilator (BiPAP; Respironics, Murrysville, PA, USA) during nocturnal sleep. The nasal-Bi-level positive airway pressure (n-BiPAP) markedly improved the central hypoventilation.

After discharge from our hospital he continued to undergo n-BiPAP during sleep at home. At 6 years of age he started the antiepileptic drugs zonisamide and sodium valproate, which were prescribed by his home doctor for paroxysmal episodes of severe anxiety and excitement. After the start of n-BiPAP treatment, his symptoms of heart failure and hypothyroidism improved, and at 7 years of age, denopamine, furosemide, spironolactone and levothyroxine sodium hydrate were all stopped (Fig. 2). The frequency of paroxysmal episodes of severe anxiety and excitement gradually decreased and there were no episodes after he turned 15 years of age. At present the patient is 20 years of age and is still undergoing n-BiPAP treatment every night during sleep without any respiratory trouble or seizures. He currently works at a vocational aid center on weekdays without any respiratory support, and leads a daily life on his own.

At 20 years of age, he underwent hypercapnic ventilation response examination involving a rebreathing technique. His ventilatory response to hypercapnia was poor (CO₂ ventilatory response: patient, 0.034 L/min/mmHg; normal range for the authors' center, 1.01–2.69), which indicated impaired chemosensitivity to hypercapnia.

Genetic studies were performed on venous samples with written informed consent from all subjects. On amplification and sequencing of the *PHOX2B* gene, the patient exhibited a 15-base pairs insertion at nucleotide 721 of the *PHOX2B* gene.¹ This in-frame insertion is predicted to give a five alanine expansion in the polyalanine tract of 20 residues in *PHOX2B* previously seen

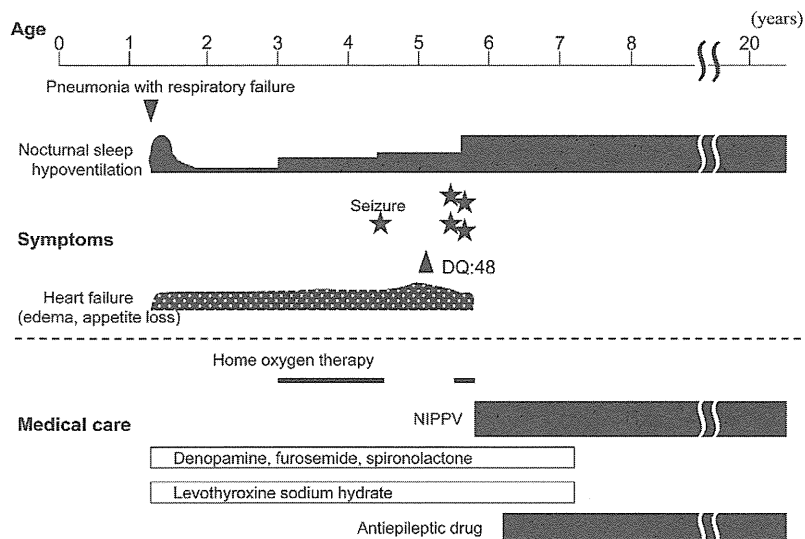


Fig. 2 Clinical course of the patient. Nocturnal sleep hypoventilation first presented at 1 year and 2 months of age with pneumonia and then gradually deteriorated as time passed. DQ, development quotient; NIPPV, nasal intermittent positive pressure ventilation.

in CCHS, which has also been reported in some cases of LO-CHS.²⁻⁵ The parents exhibited normal *PHOX2B* gene analysis results, indicating a *de novo* mutation in the patient. Being based on the clinical, physiological and genetic data we diagnosed this patient as having LO-CHS with a five-alanine expansion mutation (20/25 genotype) of *PHOX2B*.

Discussion

In this patient, it was atypical and characteristic that his nocturnal hypoventilation slowly progressed; both the frequency and severity of his sleep apnea deteriorated as time passed, and consequently he became wholly dependent on assisted ventilation for more than 4 years after the onset of the respiratory symptoms. Few papers have provided information about the changes in clinical symptoms and/or respiratory function in patients with LO-CHS with the course of time. Doherty *et al.* reported a proband with LO-CHS and his asymptomatic father who was genetically a carrier of LO-CHS. Follow-up sleep studies in the father and son after an average of 5 years revealed an elevated apnoea/hypopnoea index.² Lee *et al.* reported a Chinese family, including a proband and two daughters, who had LO-CHS. Repeated hypercapnic ventilatory response testing of the proband and one daughter showed no change in chemosensitivity, while the other daughter exhibited deteriorating chemosensitivity.⁴

n-BiPAP was chosen as the ventilatory support during sleep for our patient. The central hypoventilation resolved well with this respiratory management without considerable problems. Meanwhile, after the patient started to undergo home oxygen therapy during nocturnal sleep at 3 years of age, a paroxysmal episode of severe anxiety/excitement and seizures started around the same time. This might have been caused by carbon dioxide narcosis, which was worsened by respiratory suppression owing to the oxygen supply. One should be cautious when carrying out oxygen therapy for a suspicious LO-CHS patient. This report seeks to increase awareness as to LO-CHS by demonstrating its

various manifestations. We suggest that pediatricians should consider the possibility of LO-CHS when caring for patients with hypercapnia and/or heart failure of unknown cause.

Acknowledgments

We are indebted to Drs Akira Fukazawa (Department of Pediatrics, Hamada National Hospital), and Hiraku Doi (Department of Pediatrics, Shimane Prefectural Central Hospital) for referring the patient and providing the patient's information, and Yoshihiro Maegaki (Division of Child Neurology, Institute of Neurological Sciences, Faculty of Medicine, Tottori University) for advice regarding the preparation of this paper. We also thank Drs Kyo-ichi Yamasaki, Yukiko Nanba, Takahiro Yasumi, and Ken-itiro Watanabe (Department of Pediatrics, Matsue Red Cross Hospital) for helpful discussions.

References

- 1 Arai H, Otagiri T, Sasaki A *et al.* De novo polyalanine expansion of *PHOX2B* in congenital central hypoventilation syndrome: unequal sister chromatid exchange during paternal gametogenesis. *J. Hum. Genet.* 2007; **52**: 921-5.
- 2 Doherty LS, Kiely JL, Deegan PC *et al.* Late-onset central hypoventilation syndrome: a family genetic study. *Eur. Respir. J.* 2007; **29**: 312-6.
- 3 Weese-Mayer DE, Berry-Kravis EM, Zhou L. Adult identified with congenital central hypoventilation syndrome - mutation in *PHOX2b* gene and late-onset HCS. *Am. J. Respir. Crit. Care Med.* 2005; **171**: 88.
- 4 Lee P, Su YN, Yu CJ, Yang PC, Wu HD. *PHOX2B* mutation-confirmed congenital central hypoventilation syndrome in a Chinese family: presentation from newborn to adulthood. *Chest* 2009; **135**: 537-44.
- 5 Matera I, Bachetti T, Puppo F *et al.* *PHOX2B* mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset central hypoventilation syndrome. *J. Med. Genet.* 2004; **41**: 373-80.

I. 呼吸ケアと管理に必要な基本的知識

人工呼吸中・離脱後の気道ケアと体位管理

(3) 気管切開と加温加湿



埼玉医科大学総合医療センター 麻酔科 (教授) みやま ひでき 宮尾 秀樹 (写真) 同科 (助教) ふくやま たつや 福山 達也

国立成育医療研究センター 手術・集中治療部 (部長) すずき やすゆき 鈴木 康之

POINT

- 相対湿度、絶対湿度、飽和水蒸気量、温度の関係を正確に理解する。
- 長期気管切開カニューレ管理の加湿に関しては、エビデンスレベルの高い文献はない。
- 気管切開の加湿は、急性期は加温加湿器、亜急性期は人工鼻 (heat and moisture exchanger : HME)、慢性期は人工鼻あるいは空気開放を推奨する。
- 小児では気道が細く、加湿不足による閉塞の危険があるので注意深い観察が必要である。

はじめに

人工呼吸管理中は気管チューブによる人工呼吸中であろうが、気管切開カニューレによる人工呼吸中であろうが、加温加湿の必要性は同じです。問題は長期人工呼吸管理になる場合、気管切開を施行されることが多く、人工呼吸から離脱後の自発呼吸下でかつ気管切開中の加温加湿をいかに行うかです。本項では気管切開中で、空気による自発呼吸患者の加湿について主に記述します。また、小児の長期人工呼吸管理における加温加湿は成人と異なり、多くの問題点があるので詳述します。

空気中の湿度

「人工呼吸管理における加温加湿」の項 (p.53) で記述しましたように、病院の酸素配管や空気配管のガスや酸素ポンベのガスは湿度0%の乾燥ガスなので、加湿は必須です。一方、周りの空気はある程度の湿度を含みます。表1は温度、相対湿度の違いによる絶対湿度の早見表です。天気予報でおなじみの湿度は相対湿度ですが、同じ相対湿度でも夏と冬では絶対湿度 (1L

表1 温度と相対湿度から見る絶対湿度早見表

	相対湿度															
	30%	35%	40%	45%	50%	55%	60%	65%	70%	75%	80%	85%	90%	95%	100%	
温度	37℃	13	15	18	20	22	24	26	28	31	33	35	37	39	42	44
	36℃	12	15	17	19	21	23	25	27	29	31	33	35	37	40	42
	34℃	11	13	15	17	19	21	23	24	26	28	30	32	34	36	38
	32℃	10	12	14	15	17	19	20	22	24	25	27	29	30	32	34
	30℃	9	11	12	14	15	17	18	20	21	23	24	26	27	29	30
	28℃	8	10	11	12	14	15	16	18	19	20	22	23	25	26	27
	26℃	7	9	10	11	12	13	15	16	17	18	20	21	22	23	24
	24℃	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22
	22℃	6	7	8	9	10	11	12	13	14	15	16	17	18	18	19
	20℃	5	6	7	8	9	10	10	11	12	13	14	15	16	16	17
	18℃	5	5	6	7	8	9	9	10	11	12	12	13	14	15	15
	16℃	4	5	5	6	7	8	8	9	10	10	11	12	12	13	14
	14℃	4	4	5	5	6	7	7	8	9	9	10	10	11	12	12
	12℃	3	4	4	5	5	6	6	7	8	8	9	9	10	10	11
	10℃	3	3	4	4	5	5	6	6	7	7	8	8	9	9	9
	8℃	2	3	3	4	4	5	5	5	6	6	7	7	7	8	8
	6℃	2	3	3	3	4	4	4	4	5	5	5	6	6	7	7
4℃	2	2	3	3	3	4	4	4	4	5	5	5	5	6	6	
2℃	2	2	2	3	3	3	3	3	4	4	4	4	5	5	6	
0℃	1	2	2	2	2	3	3	3	3	3	4	4	4	4	5	

単位は mg/L。小数点以下は四捨五入。表の□(相対湿度 100%) はその温度における飽和水蒸気量を表す。

の空気に含まれる水蒸気量) が異なります。表1の50%の所を見てください。冬の4℃、50%の空気は3 mg/Lの水蒸気しか含まないのに対し、夏の30℃、50%の空気は15 mg/Lと3倍量の水蒸気を含みます。気道の湿度を論じる時には、温度と相対湿度と絶対湿度の関係を正確に理解する必要があります。

「人工呼吸における加温加湿」の項で記述したように、相対湿度と絶対湿度の関係式は

$$\text{相対湿度 (\%)} = \frac{\text{絶対湿度 (mg/L)}}{\text{飽和水蒸気量 (mg/L)}} \times 100$$

です。

式の飽和水蒸気量^①が表1の右端の値であり、温度により変化します。

例えば、4℃、50%の空気は表1から3 mg/Lの絶対湿度をもちます。4℃の飽和水蒸気は表1の右端から6 mg/Lとなり、

$$50 (\%) = \frac{3 (\text{mg/L})}{6 (\text{mg/L})} \times 100$$

したがって、温度、相対湿度、絶対湿度の3つ因子のうち、2つがわかれば、表1からもう一つもわかります。

① 飽和水蒸気量
空気1L中に含まれる水蒸気量の最大限度で、温度で一意的に決まり、温度が高いほど大きくなる。

気管内の温度湿度

「人工呼吸管理における加温加湿」の項でも示した図1をもとに解説します。気管支の第2分岐あたりが、体温下で相対湿度は100%になるといわれています。体温を37℃とすると絶対湿度は44 mg/Lとなります(表1右上)。外気が22℃で相対湿度が50%の場合、表1から絶対湿度(水蒸気含量)は10 mg/Lです。この外気が気管切開孔から肺内に入って肺胞まで到達する間に37℃まで体温で暖められながら、周りの粘膜から水分を奪取しながら44 mg/Lの絶対湿度となります。この44 mg/Lと外気の10 mg/Lの差である34 mg/Lの水蒸気は気管切開孔から気管支第2分岐くらいまでの間で、気管粘膜から奪取されます。通常、気管粘膜からは絶えず粘液が分泌されていて、その粘液を気管粘膜の線毛により上気道へ送りながら気道の清浄をはかっています。この粘液から水分が奪われると、痰の粘稠化が起こり、線毛も機能不全となり、気道の清浄化運動が破綻します。これが、無気肺²⁾、肺炎などの肺合併症を起こす基礎状態を作ることになります。極端な場合は粘液が固形化し、気道閉塞となる場合もあります。

② 無気肺

肺胞の一部が虚脱し換気ができていない状態を指す。粘膜炎により、小気道が閉塞し、その領域の肺胞が虚脱し無気肺になる。低酸素血症の原因となる。

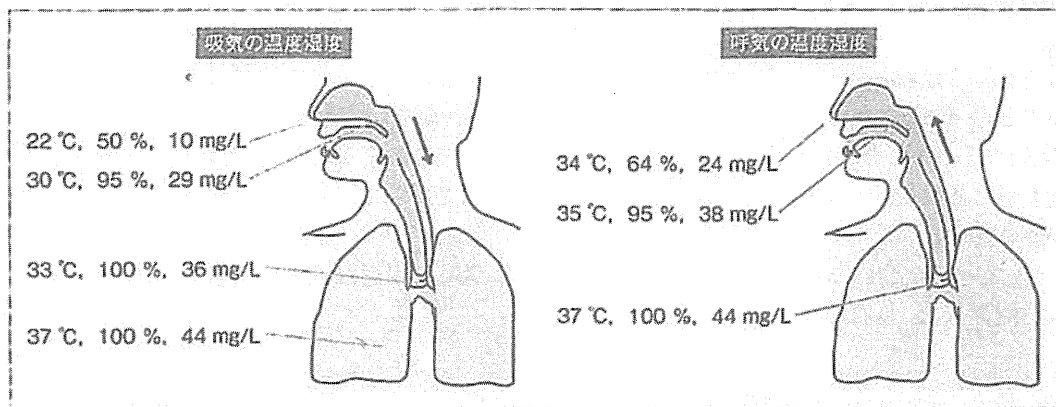


図1 22℃の大気を呼吸している場合の健康人の気道の温度湿度分布 (文献1を参照して作成)

加温加湿器か人工鼻か何もしないか

現在、長期気管切開カニューレ管理の加湿に関しては、エビデンスレベルの高い文献はありません。人工鼻 (artificial nose, heat and moisture exchanger : HME) にはいろいろありますが、麻酔中に使用するタイプや気管切開カニューレ用のタイプもそれぞれあります(図2)。死腔の大きいタイプの人工鼻(図3)は死腔がコンデンサーの役割を果たし、加湿性能がよく、遊びがあるため、頸部の動きにも追従しやすいです。気管切開患者の長期ケアの論文²⁾では、「成人長期気管切開患者の加湿に関しては、個々の施設のローカルルールで対処している。脱水がなく、水分摂取も普通で、全身状態