研究成果の刊行に関する一覧表

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Hasegawa H, Kawasaki K, Inoue H, Umehara M, Takase M	Epidemiologic survey of patients with congenital central hypoventilation syndrome in Japan	Pediatr Int.	54	123-126	2012
Fujiwaki T, Hasegawa H, Arai H, Hayasaka K, Ohta S.	Slowly progressive sleep apnea in late-onset central hypoventilation syndrome.	Pediatr Int.	54	290-292	2012
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ORIGINAL ARTICLE

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

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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.
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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 polyalanine 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, Bachetti *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-CHS) 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-CHS, 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 polyalanine sequence in exon 3 from genomic DNA with primers 22F 5′-GCACTGACCCGGACAGCACT-3′ and 3.3R 5′-TACCCGCTCGCCCA

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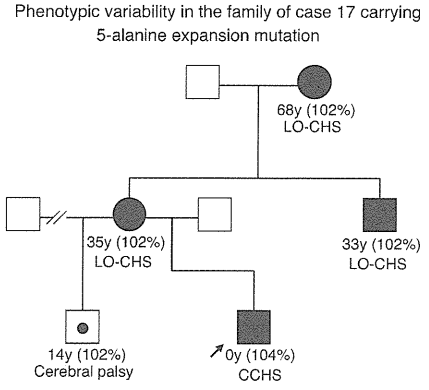

Figure 1 Phenotypic variability in the family of case 17 carrying 5-alanine expansion mutation. Filled squares and circles indicate affected males and females, respectively. Numbers under symbols and those in parentheses represent the age and percentage of mosaicism, respectively. The arrow indicates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

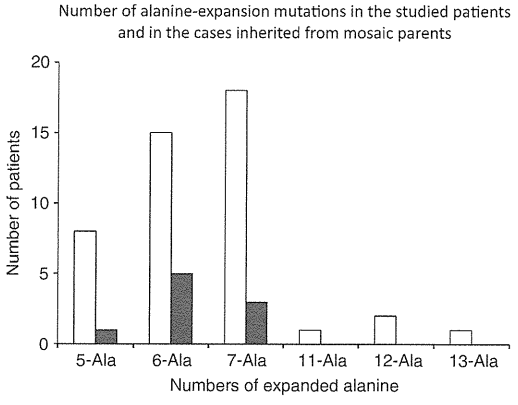

Figure 2 Number of alanine-expansion mutations in the studied patients and in the patients inherited from mosaic parents. Open and filled bars represent the studied patients and patients inherited from the mosaic parents, respectively.

CTCG-3′ or with primers 22F 5′-GCACTGACCCGGACAGCACT-3′ and 279R 5′-GAGCCCAGCCTTGTCCAGG-3′; the forward probes were 5′ labeled with carboxyfluorescein (FAM) according to the method of Trochet *et al.*[5,9] and Bachetti *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 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 R^2 coefficient. The equation of the regression line and R^2 coefficient for 5-, 6-, 7- and 13-alanine expansion mutation were $Y=0.49X$ ($R^2=0.9948$), $Y=0.49X$ ($R^2=0.9998$). $Y=0.48X$ ($R^2=0.9986$) and $Y=0.46X$ ($R^2=0.9977$), 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.

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).
[a]7-alanine expansion was speculated from his father's data.

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.

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Slowly progressive sleep apnea in late-onset central hypoventilation syndrome

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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, SpO_2 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 SpO_2 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

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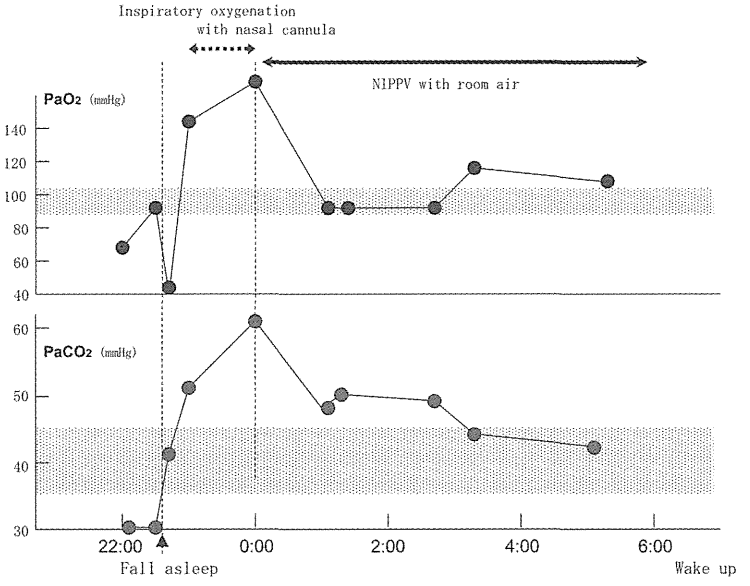

Fig. 1 Blood gas analysis during night sleep with inspiratory oxygenation and nasal intermittent positive pressure ventilation (NIPPV) in a hospital at 5 years and 10 months of age. The upper and lower panels show the time courses of PaO_2 and $PaCO_2$, respectively. As soon as the patient fell asleep, PaO_2 decreased and $PaCO_2$ increased immediately. Following inspiratory oxygenation with a nasal cannula, PaO_2 increased in response to it, while $PaCO_2$ continued to increase further. Subsequent NIPPV with room air improved both PaO_2 and $PaCO_2$ successfully until he woke up. The shadowed areas indicate the normal ranges for PaO_2 and $PaCO_2$, 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 fiberscopic 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 (CO_2 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

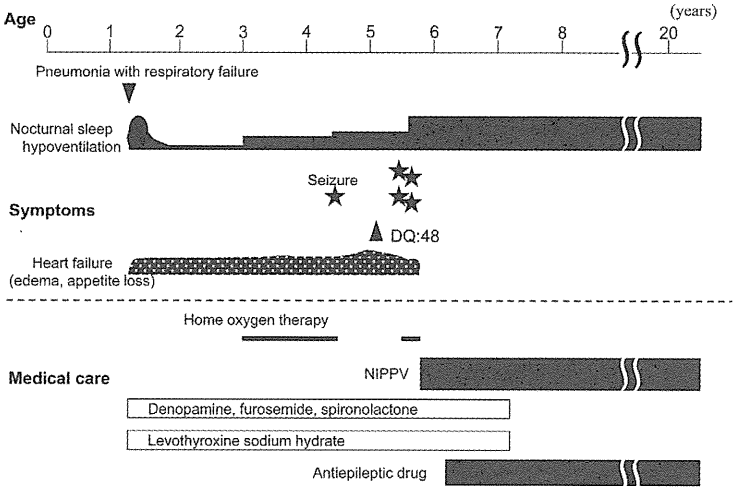

Fig. 2 Clinical course of the patient. Nocturnal sleep hypoventilation first presented at 1 year and 2 months of age with pneumonia and then gradually deteriorated as time passed. DQ, development quotient; NIPPV, nasal intermittent positive pressure ventilation.

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

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Original Article

Epidemiologic survey of patients with congenital central hypoventilation syndrome in Japan

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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 CO_2 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.

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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 bodyweight 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

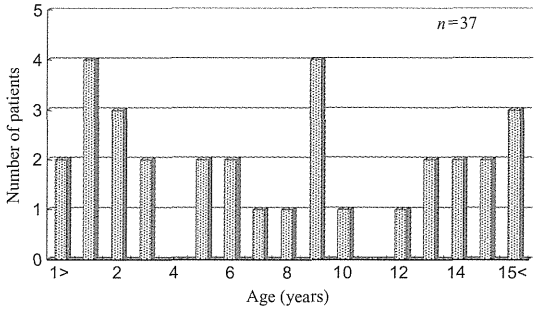

Fig. 1 Age distribution of patients with congenital central hypoventilation syndrome (CCHS).

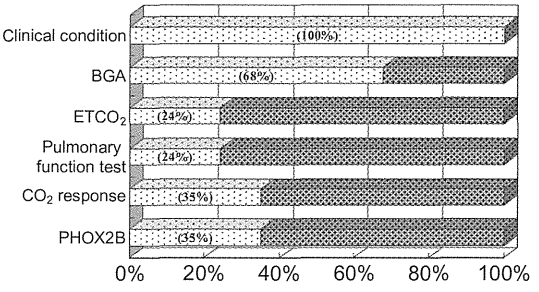

Fig. 3 Diagnosis-related examinations. BGA, blood gas analysis; CO_2 response, ventilatory response to inhaled CO_2; $ETCO_2$, end-tidal CO_2; PHOX2B, paired-like homeobox gene 2B.

(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 CO_2 (14/37, 35%); genetic analysis, namely *PHOX2B* analysis (13/37, 35%); pulmonary function test (9/37, 24%); and end-tidal CO_2 measurement (29/27, 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, 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-

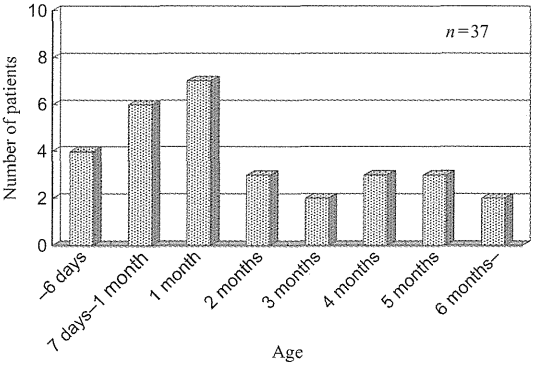

Fig. 4 Age at diagnosis.

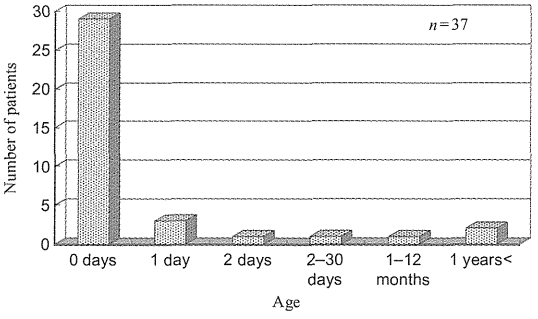

Fig. 2 Age at onset.

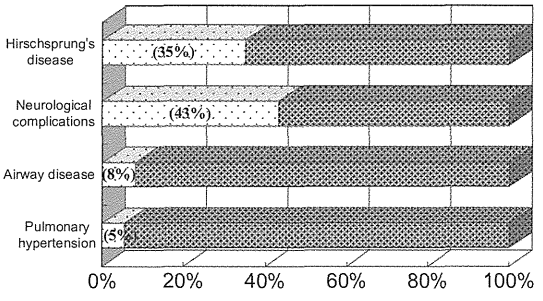

Fig. 5 Background diseases and complications.

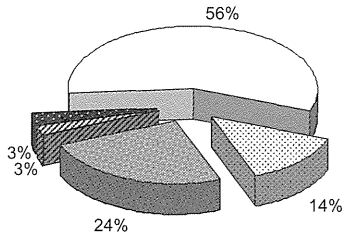

Fig. 6 Ventilation methods. □, ventilation via tracheotomy; ▨, facemask ventilation; ▩, nasal-mask ventilation; ▨, diaphragmatic pacers; ■, ventilation via intratracheal tube.

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 (l6/22, 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.

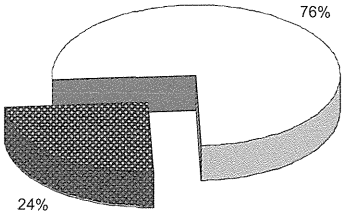

Fig. 7 Ventilatory support. □, sleep ventilation (n = 28); ■, 24-h ventilation (n = 9).

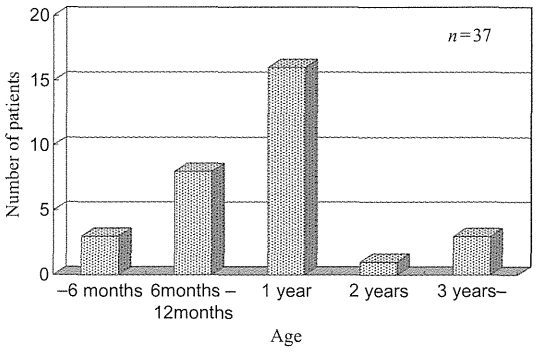

Fig. 8 Age at discharge.

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%).

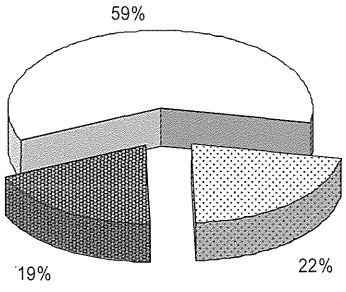

Fig. 9 Prognoses of patients with congenital central hypoventilation syndrome. □, survival without handicap; ▨, survival with handicap; ■, death.

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.

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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，CO_2を低下させる効果は期待できないため，CO_2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endotrachial 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
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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 system[TM]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 cmH_2O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 cmH_2O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CO_2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①1回換気量が死腔量より少ない，②換気回数が3Hz以上，③active 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，$PaCO_2$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。PaO_2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)[1]。低いMAPでHFOを行うと振動が肺胞でなく末梢気道に直接作用し，気道損傷の原因となる。5 cmH_2OではHFOの効果はなく，6 cmH_2Oで間質性肺気腫が多発することから，最低でも7 cmH_2OのMAPは保つようにする。

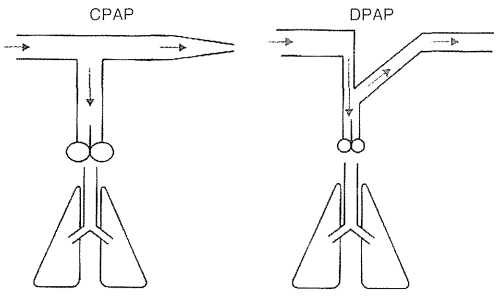


図1　CPAPとDPAP

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 technique[7]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

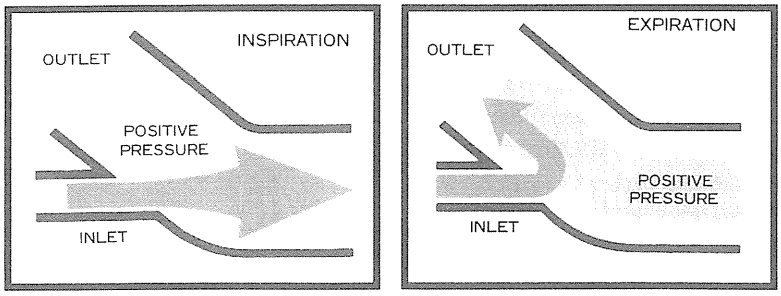


図2　Nasal DPAPによるコアンダ効果

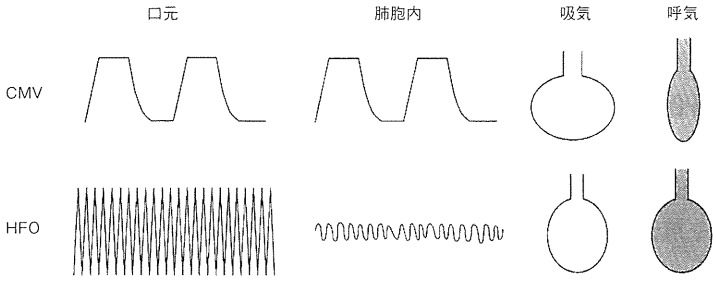


図3　CMVとHFO

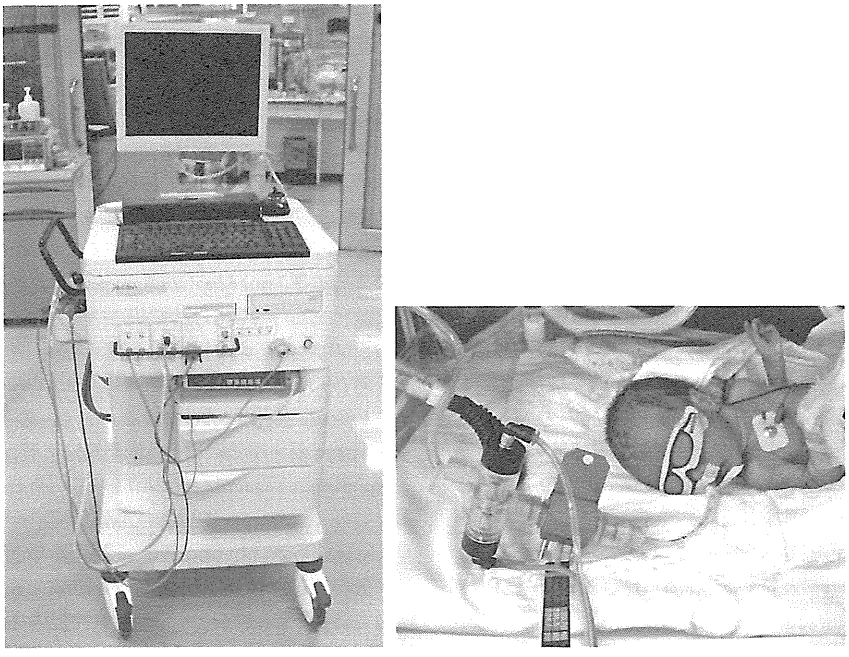


図4　アイビジョン社製呼吸機能測定装置

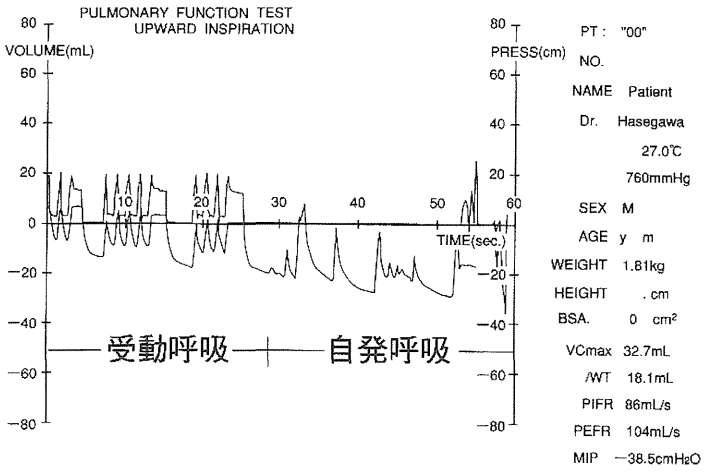


図5　呼吸機能測定画面
上側が圧曲線，下側が換気量曲線。前半は受動呼吸，後半は自発呼吸による測定。

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気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。前半の受動呼気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/cmH_2O/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

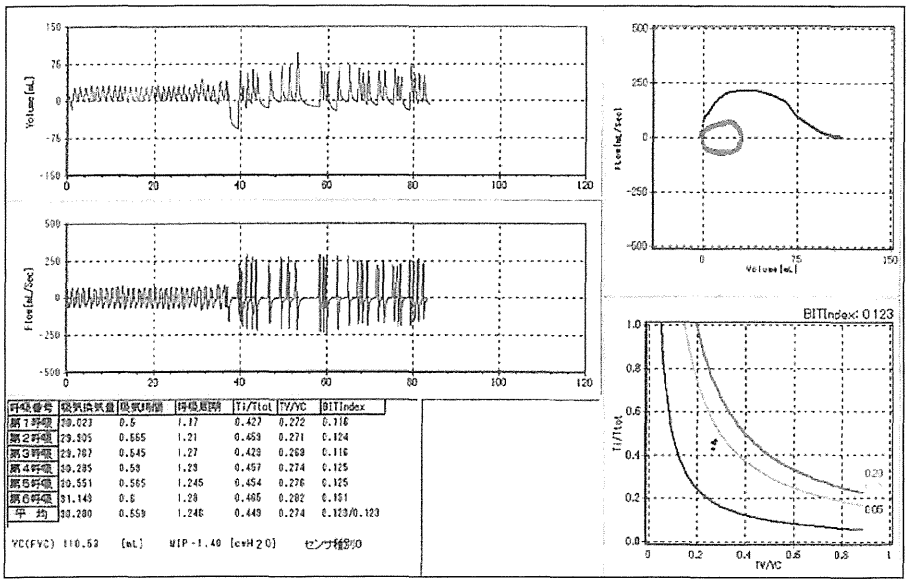
新生児の呼吸器疾患は疾患ごとに特徴的な呼吸機能を呈する。胸部X線などと組み合わせることにより，より的確な診断が可能となる。各疾患の呼吸機能の特徴を**表1**に示す。

(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(Ttot)に対する吸気時間(Ti)の割合(Ti/Ttot)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＝(Ti/



呼吸番号	吸気換気量	吸気時間	呼吸周期	Ti/Ttot	TV/VC	BITIndex
第1呼吸	30.023	0.5	1.17	0.427	0.272	0.116
第2呼吸	29.935	0.555	1.21	0.459	0.271	0.124
第3呼吸	29.787	0.545	1.27	0.428	0.269	0.115
第4呼吸	30.285	0.58	1.29	0.457	0.274	0.125
第5呼吸	30.551	0.565	1.245	0.454	0.276	0.125
第6呼吸	31.143	0.6	1.28	0.466	0.282	0.131
平 均	30.280	0.559	1.246	0.449	0.274	0.123/0.123

VC(FVC) 110.53 [mL] MIP -1.49 [cmH2O] センサ種別0

図6 BITIの測定・解析画面

Ttot)×(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，吸気努力の終了を関知すると自動的に弁を開放し安静呼吸に戻す。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：Er)，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。検査時間や児にかける負担などから，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)。中枢性肺胞低換気症候群(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[8)]

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，気道病変が疑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

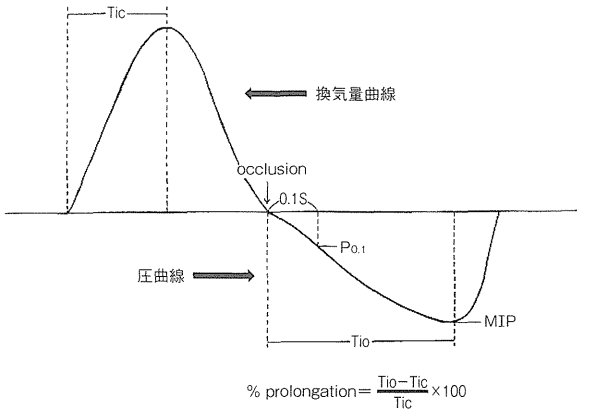


図7　反射性中枢性呼吸機能検査(気道閉塞法)

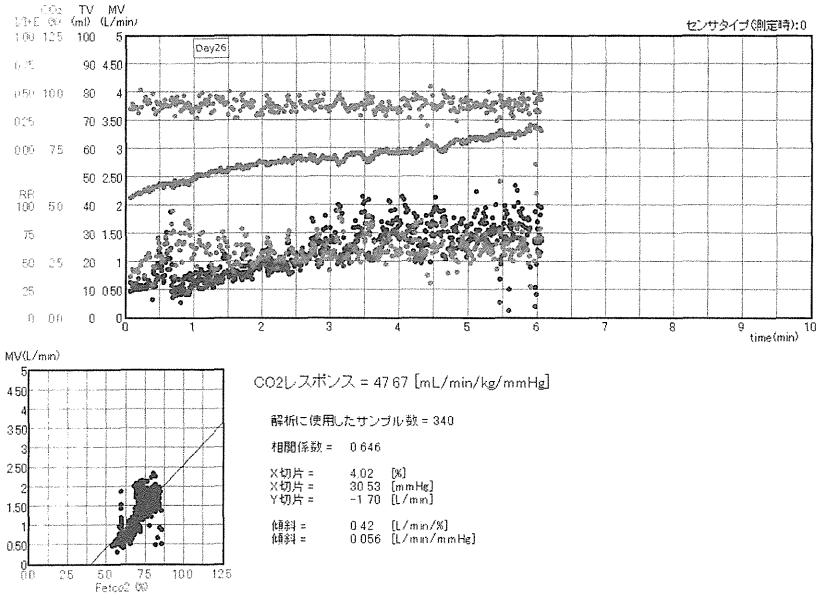


図8　化学性中枢性呼吸機能検査(CO_2 response)の測定・解析画面

児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Roberts[9]，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

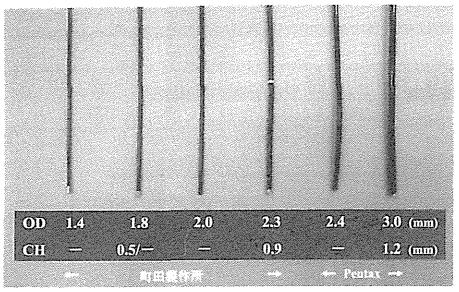


図9 新生児・小児用気管支ファイバースコープ

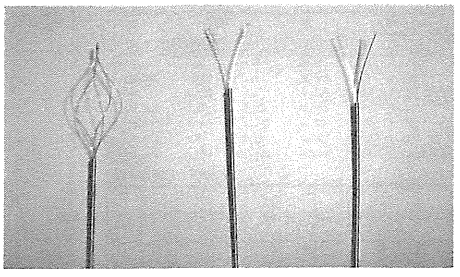


図10 新生児・小児気道処置用鉗子
バスケット鉗子(左)，2本爪鉗子(中)，3本爪鉗子(右)

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，NO_2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20 ppmで開始。開始後4時間は20 ppmを維持し，酸素化の改善に伴い5 ppmに減量する。投与中止は，吸入濃度を1 ppmまで徐々に減量し，問題なければ，F_{IO_2}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。

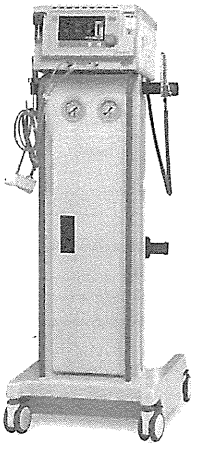


図11 INOvent delivery system

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。

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