PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2014) **56**, 227–229 doi: 10.1111/ped.12247

Original Article

Breathing intolerance index in healthy infants

Hisaya Hasegawa, Nobuhide Henmi, Shio Tsuruta, Yoshitaka Miyoshi, Yousuke Yamada, Junichi Muto and Masanori Wasa
Division of Neonatal Intensive Care, Tokyo Women's Medical University Medical Center East, Tokyo, Japan

Abstract ***Background***: The breathing intolerance index (BITI) is used to justify ventilator use in adult patients with pulmonary or chest wall disease. BITI in ventilated patients is mostly ≥0.15. The mean BITI of healthy adults in the upright sitting position and the supine position is 0.050 ± 0.009 and 0.057 ± 0.016, respectively. The aim of this study was to establish a normal BITI in infants.

Methods: Thirty healthy infants were examined in the supine position. BITI was defined as BITI = (Ti/Ttot) × (TV/VC), where Ti is inspiratory time, Ttot is total breath cycle duration, TV is tidal volume, and VC is vital capacity. TV and Ti/Ttot were obtained from tidal breathing at rest and VC was obtained spirometrically. BITI was calculated using customized software.

Results: The mean ± SD BITI of 30 healthy infants in the supine position was 0.120 ± 0.013. The BITI of infants was significantly higher than that of adults.

Conclusions: BITI was measured in healthy infants. Further evaluation is needed to determine the significance and usefulness of BITI.

Key words: breathing intolerance index, infant, pulmonary function.

Bellemare and Grassino identified a relationship between the tension time index of the diaphragm (TTIdi) and diaphragm endurance.[1] They noted that the ability to sustain the transdiaphragmatic pressure swings that are required for continuous autonomous breathing is limited to <45 min when TTIdi is >0.15. TTIdi is the product of the mean transdiaphragmatic pressure swing divided by the maximum transdiaphragmatic pressure (Pdi/PdiMax) and the inspiratory time divided by total breath time (Ti/Ttot). Koga *et al.* substituted the tidal volume divided by the vital capacity (VT/VC) for Pdi/PdiMax in the Bellemare and Grassino relationship.[2] Koga *et al.* named this new index the breathing intolerance index (BITI). Koga *et al.* showed that the BITI in ventilated patients is mostly ≥0.15. The aim of this study was to establish a normal BITI in healthy infants.

Methods

Thirty healthy infants were examined in the supine position. There were 16 boys and 14 girls. The mean gestational age was 39.4 ± 1.2 weeks (range, 37.6–40.9 weeks). The mean birth bodyweight was 3033 ± 341 (range, 2530–4034 g). The mean age at the time of measurement was 8.4 ± 7.1 days (range, 0–25 days). BITI was defined as BITI = (Ti/Ttot) × (TV/VC). BITI was calculated using a spirometer to analyze flow and volume signals in order to measure Ti, Ttot, TV, and VC. TV and Ti/Ttot were obtained from tidal breathing at rest and VC was obtained while crying when the sole of the foot was stimulated spirometrically using a pulmonary function measuring system (ARFEEL; Aivision, Tokyo, Japan) and face mask (Fig. 1). BITI was calculated using customized software and was displayed on the PC monitor. Each subject had two sessions: one for baseline values and the other for VC. The most consistent six consecutive TV waveforms at rest were averaged for Ti, Ttot, and TV. VC was defined as the maximum volume observed (Fig. 2). Non-paired Student's t test was used to compare the data. $P < 0.05$ was used to denote statistical significance.

This study was approved by the Tokyo Women's Medical University Research and Ethics Committees. Written informed consent was obtained from the parents.

Results

All examinations were performed without complications. The mean BITI of 30 healthy infants in the supine position was 0.120 ± 0.013. There was no significant relationship ($r = -0.087$) between age and BITI (Fig. 3).

The mean BITI of 21 healthy adults in the upright sitting position and in the supine position was 0.050 ± 0.009 and 0.057 ± 0.016, respectively.[2] The BITI of infants was significantly higher than that of adults (Table 1). The mean Ti/Ttot in healthy infants was significantly higher than that of healthy adults in the supine position (0.450 ± 0.048 vs 0.416 ± 0.055, respectively). The mean TV/VC in healthy infants was also significantly higher than that of healthy adults in the supine position (0.271 ± 0.049 vs 0.138 ± 0.035, respectively). The difference in TV/VC had a greater influence on the difference in BITI compared to Ti/Ttot.

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: hisayani@dnh.twmu.ac.jp

Received 30 January 2013; revised 8 August 2013; accepted 9 October 2013.

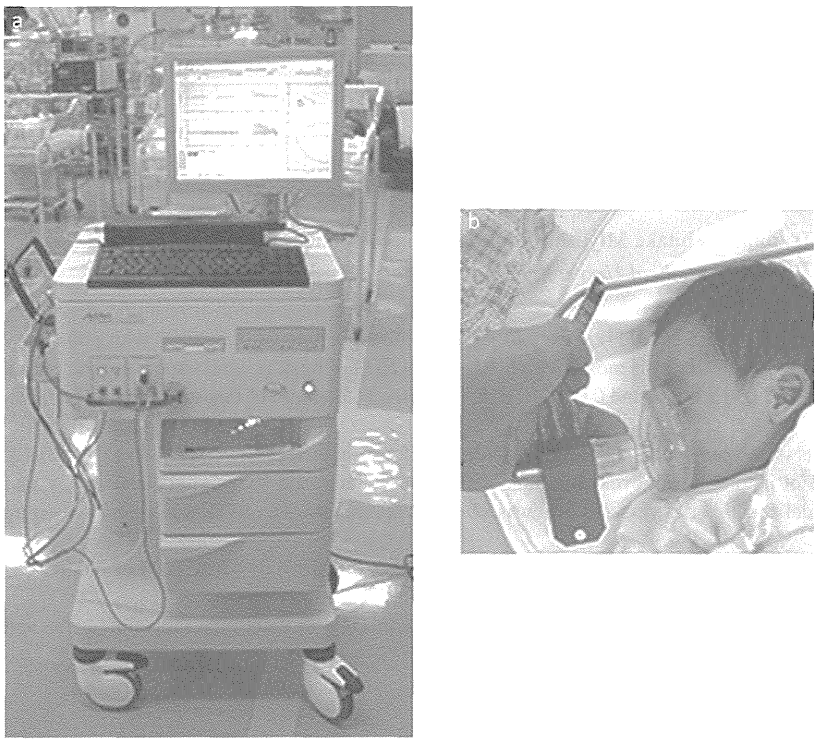


Fig. 1 (a) Aivision pulmonary function measuring system and (b) pneumotachometer with mask.

Discussion

For the evaluation of respiration, a wide range of indexes is used, such as respiratory rate, rapid shallow breathing index, maximum inspiratory pressure, and partial pressure of carbon dioxide in arterial blood, but they have been reported to be unreliable for evaluation in the acute care setting.[3–6] Also, use of VC alone to determine the need for ventilator use has been reported as inaccurate.[7,8]

The increase in the work of breathing leads to muscle fatigue. Because the diaphragm mainly contracts during inspiration, it can become exhausted more rapidly at any given tension if Ti/Ttot is abnormally increased. The diaphragm can also become exhausted more rapidly at any given Ti/Ttot if the Pdi/PdiMax is greater than normal. Bellemare and Grassino identified a relationship between the tension time index of the diaphragm (TTIdi) and diaphragm endurance (Tlim): Tlim = 0.1 $(TTIdi)^{-3.6}$.[1] But the

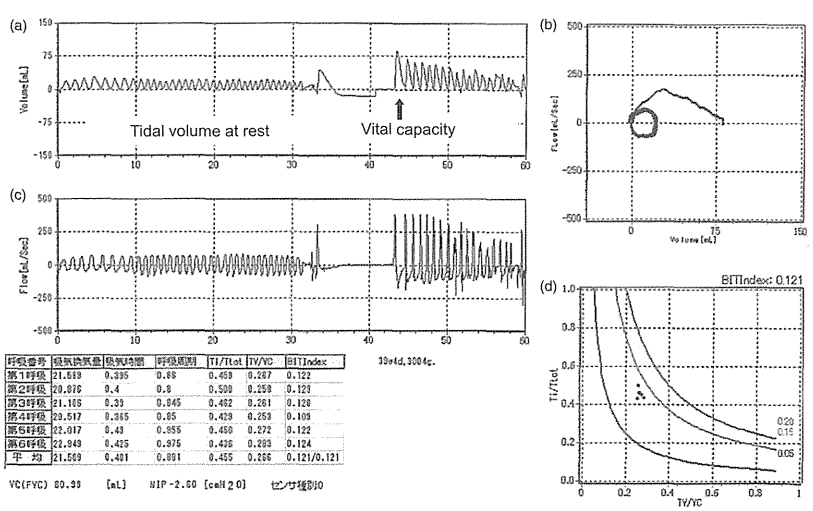


Fig. 2 (a–c) Volume, flows, and (d) breathing intolerance index (BITI). The six consecutive breath-by-breath data are plotted on the graph and averaged to obtain a BITI of 0.121.

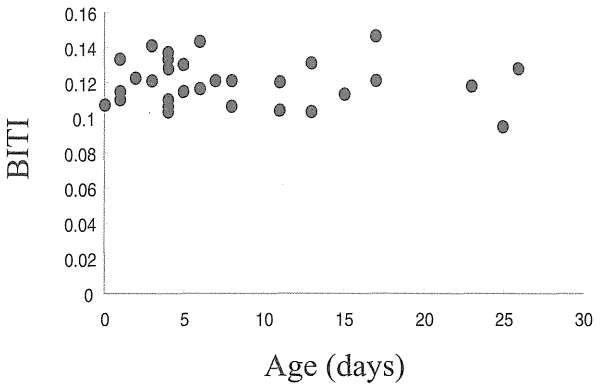


Fig. 3 Breathing intolerance index (BITI) vs age in healthy infants in the supine position.

TTIdi takes into account the exhaustion of only the diaphragm, so the BITI may be more useful because it expresses the endurance of all of the respiratory muscles. The BITI of infants was significantly higher than that of adults. Because this BITI is a physiologic constant, there seems to be no distinction between infants and adults. Infants are considered to have less reserved power in respiration compared to adults.

Table 1 BITI in the supine position

	Adults ($n = 21$)[2]	Infants ($n = 30$)	P
BITI	0.057 ± 0.016	0.120 ± 0.013	<0.0001
Ti/Ttot	0.416 ± 0.055	0.450 ± 0.048	0.025
TV/VC	0.138 ± 0.035	0.271 ± 0.049	<0.0001
TV/kg (mL/kg)	8.4 ± 3.0	6.4 ± 1.6	0.001
VC/kg (mL/kg)	61.0 ± 12.7	23.4 ± 3.7	<0.0001

BITI, breathing intolerance index; Ti, inspiratory time; Ttot, total breath cycle duration; TV, tidal volume; VC, vital capacity.

Bach *et al.* hypothesized that a new index, the ventilator requirement index (VRI), which reflects ongoing inspiratory muscle action rather than effort over only one breath cycle, might better correlate with symptomatic inspiratory muscle dysfunction.[9] Bach *et al.* defined VRI as (Ti/Ttot × TV/VC) multiplied by respiratory rate, or 60 × Ti/(Ttot)^2 × TV/VC (an equivalent equation). In the future, it may be necessary to consider the use of VRI in infants.

We measured BITI in healthy infants. Further evaluation is needed to determine the significance and usefulness of BITI.

References

1 Bellemare F, Grassino A. Effect of pressure and timing of constriction on human diaphragm fatigue. *J. Appl. Physiol.* 1982; **53**: 1190–95.
2 Koga T, Watanabe K, Sano M *et al.* Breathing intolerance index, a new indicator for ventilator use. *Am. J. Phys. Med. Rehabil.* 2006; **85**: 24–30.
3 Pierson DJ. Indication for mechanical ventilation in adults with acute respiratory failure. *Respir. Care* 2002; **47**: 249–65.
4 Wedzicha JA, Muir JF. Noninvasive ventilation in chronic obstructive pulmonary disease, bronchiectasis and cystic fibrosis. *Eur. Respir. J.* 2002; **20**: 777–84.
5 AARC. Consensus statement: Noninvasive positive pressure ventilation. *Respir. Care* 1997; **42**: 365–9.
6 International Consensus Conferences in Intensive Care Medicine. Noninvasive positive pressure ventilation in acute respiratory failure. *Am. J. Respir. Crit. Care Med.* 2001; **163**: 283–91.
7 Bellemare F, Grassino A. Force reserve of the diaphragm in patients with chronic obstructive pulmonary disease. *J. Appl. Physiol.* 1983; **55**: 8–15.
8 Gelinas D. Nocturnal oximetry as an early indicator of respiratory involvement in ALS: Correlation with FVC plus symptoms and response to NIPPV therapy. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 2000; **1**: 38–9.
9 Bach JR, Goncalves M, Eisenberg M *et al.* A ventilator requirement index. *Am. J. Phys. Med. Rehabil.* 2008; **87**: 285–91.

PEDIATRICS INTERNATIONAL Official Journal of the Japan Pediatric Society

Pediatrics International (2014) ••, ••–••

doi: 10.1111/ped.12360

Original Article

Decreased granulomatous reaction by polyurethane-coated stent in the trachea

Hikoro Matsui,[1] Takehiko Hiroma,[2] Hisaya Hasegawa[4] and Yoshifumi Ogiso[3]

[1]*Intensive Care Unit, Departments of* [2]*Neonatology,* [3]*Pathology, Nagano Children's Hospital, Nagano and* [4]*Department of Neonatology, Tokyo Women's Medical University Medical Centre East, Tokyo, Japan*

Abstract ***Background***: Reducing granulomatous reaction for stent implantation is important for the treatment of tracheobronchomalacia because formation of granuloma leads to refractory complication causing further respiratory distress. The purpose of this study was to clarify granulomatous reaction of newly innovated coated stents compared to non-coated metal stents.

Methods: Materials and animal experiments were performed using the newly invented metallic stent (LASER-cut stainless steel with a coating of polyurethane). In the materials experiment, the correlation between the holding force and deformity was tested by a compressor. In the animal examination, coated stents were orally implanted into the trachea in five rabbits, while non-coated stents were implanted in another five rabbits. After 3 weeks' observation, the inner diameter was measured by 3-D computed tomography, and the number of granulation tissues was counted by bronchofiberscope. Histological investigation followed in both groups.

Results: In the materials experiment, new stents demonstrated a holding force similar to stainless steel stents. In the animal experiment, no difference was found in the inner diameter of the coated and non-coated stent groups (5.70 ± 0.17 vs 5.60 ± 0.27, $P = 0.07$). However, the number of granulation tissues was higher in non-coated stents than in coated stents (1.60 ± 0.55 vs 0.40 ± 0.55, $P < 0.01$). Histological investigation showed direct attachment of metal to the tracheal wall around the non-coated stents where epithelial structure was destroyed, while tracheal epithelia were preserved in the group of coated stents.

Conclusions: The new polyurethane-coated metallic stent maintains enough holding force, and reduces histobiological reaction to foreign bodies in this experiment.

Key words airway, collapse, granulation tissue, stent implantation, tracheobronchomalacia.

Severe tracheobronchomalacia (TBM) causes severe airway obstruction due to the structural weakness of trachea or bronchi in children causing refractory respiratory failure.[1] In patients with TBM, the airway is functionally collapsed by positive intrathoracic pressure, resulting in respiratory deterioration. Further respiratory efforts frequently cause airway collapse and a weakening of wall structures in the airway. A significant number of sudden deaths sometimes occur after violent crying or coughing, attributable to airway collapse.[2,3]

Stent implantation (SI) in the trachea is a controversial treatment in daily clinics, although metallic stents are relatively easy to implant. Formation of granuloma is the most common complication with SI, causing other progressive stenoses as a result of histological reaction between tracheal epithelia and the implanted stent.[4] Metal on epithelia results in histological inflammation, which in turn causes a granulomatous reaction with organized collections of macrophages in the interstitial tracheal tissue. This excessive reaction to contact with metal causes the paradoxical result of respiratory deterioration, given the fact that SI is inserted into the trachea to reduce airway obstruction. Thus, tracheal SI is not a favorable choice for treatment of TBM yet.

Therefore, we devised a metallic tracheal stent coated with a less irritating material to reduce the granulomatous reaction of tracheal epithelia. We hypothesized that an implanted metallic stent, enveloped in polyurethane, would provoke little histological reaction to tracheal epithelia and result in less granuloma. In this study, we tested the material properties of the new tracheal stent, and then we confirmed the long-term geographical and histological findings after SI in animals.

Methods

We performed materials and animal experiments using newly developed coated stents. The main framework of the stent is made from stainless steel cut by LASER. The coating material is polyurethane, 100 μm in thickness.

Correspondence: Hikoro Matsu, MD PhD, Department of Intensive Care Unit, Nagano Children's Hospital, 3100 Toyoshina, Azumino City, Nagano 3998288, Japan. Email: hikoromatsui@googlemail.com

Received 13 March 2013; revised 29 October 2013; accepted 10 April 2014.

Material experiments

To investigate the stents' holding force, a balloon was used to compress 10-mm- and 20-mm-long coated stents to 6 mm in diameter. The holding force (Newton Unit) was measured with deformity from 0% to 50%, in 10% steps using a compressor (AUTOGRAPH, AG-500D, Shimazu, Kyoto, Japan) (Fig. 1). Deformity was defined by the following formula:

$$\text{Deformity (\%)} = (\text{Stent external diameter without compression} - \text{Stent external diameter}) \times 100 / \text{Stent external diameter without compression.}$$

The deformity/holding force correlation of the coated stents was compared with two other commercially available stents, a PALMAZ stent (Johnson and Johnson, New Brunswick, NJ, USA, balloon-expandable stent made from stainless steel: 6 mm in diameter and 20 mm or 10 mm in length) and a LUMINEX stent (BARD, Murray Hill, NJ, USA; self-expandable, made from Nitinol: 6 mm in diameter and 20 mm in length).

Animal experiments

Ten wild adult Japanese rabbits were used in this study (bodyweight: 2.5–3.0 kg). All rabbits were sedated, orally intubated with a silicone tube (3.5 mm in diameter, Portex tube, Smith Medical, Saint Paul, MN, USA) using a straight laryngoscope. Stents (10 mm in length) were mounted centrally on a non-compliant balloon (3.5 mm in diameter and 20 mm in length) for implantation. All rabbits breathed spontaneously during procedures.

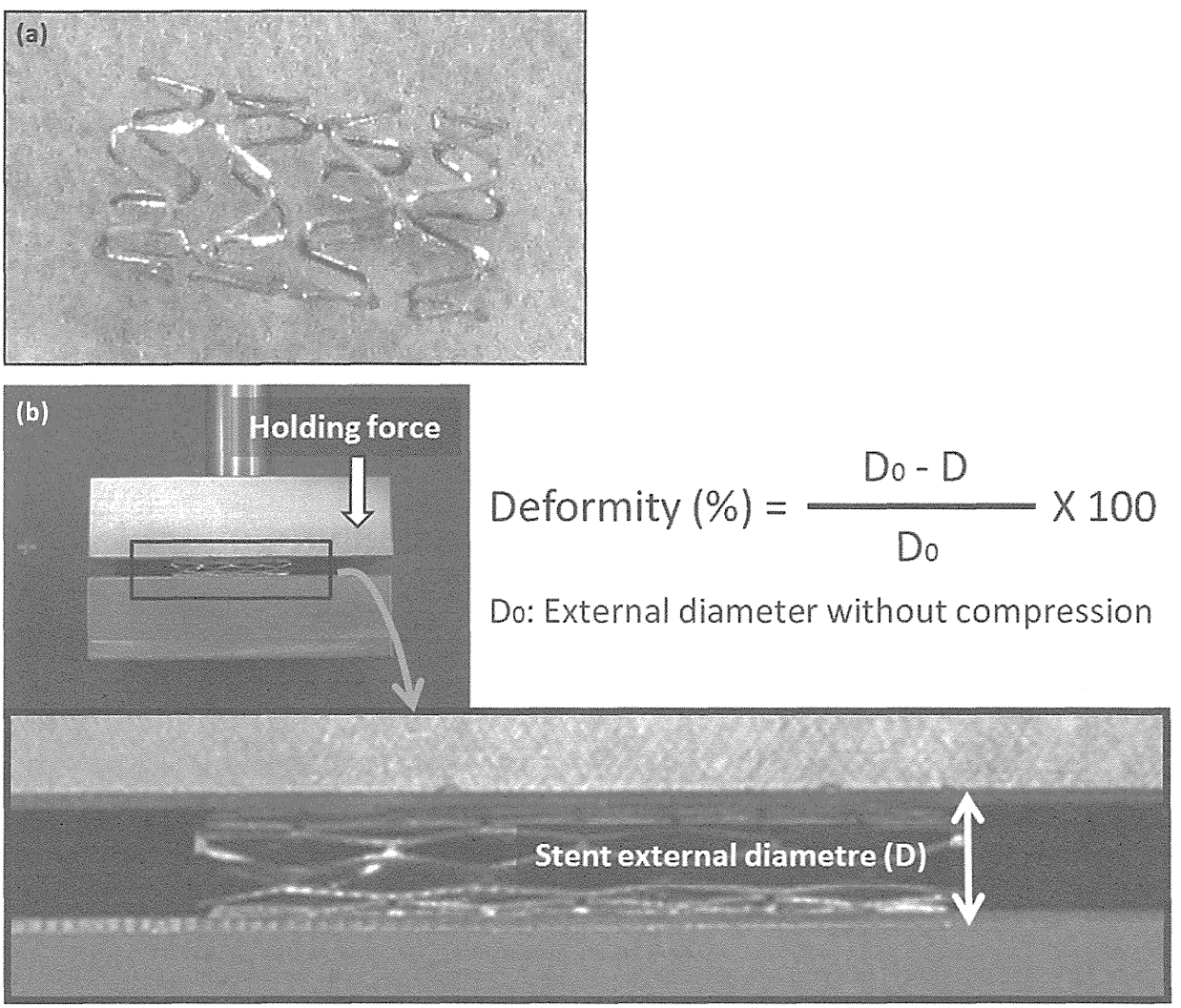


Fig. 1 New metallic stent and examination of holding force by compressor. (a) Picture of metallic stent. (b) Compressor and formula deformity. Deformity was defined as shortening as a fraction of external diameter of the stent. Holding force was measured in Newton units.

We implanted coated stents in five of the ten rabbits (group A) and non-coated stents in the other five (group B). Pressure during balloon dilatation for SI was 10 atmospheres. SI was performed immediately after intubation into the middle of the trachea of each rabbit. The airway tube was quickly removed after SI, and the rabbits were observed for 3 weeks.

After the observation period, all rabbits were humanely put down, with a lethal pentobarbital injection. The trachea and main bronchi were then removed and fixed by formalin. Specimens were then assessed. First, we examined multi-detector row computed tomography (CT) with the thickness of 1 mm to assess 3-D structure of the frame. Volume-rendered CT images were used to create a 3-D reconstructed image. Deformation was also checked for visually. The diameter of the inner-frame cavity at both the ends and the middle of the stents were measured and compared between groups A and B. Second, a bronchofiberscope was used to investigate the internal cavity of the implanted stents. Protrusions of granulation tissue in the tracheal cavity were observed and counted, before comparing the count between groups A and B. Then, specimens were embedded into epoxy resin, followed by sectioning for histological investigation with hematoxylin–eosin stain.

Analysis was performed using SPSS (SPSS, Chicago, IL, USA). Non-paired *t*-test was used for comparison of each value between group A and group B, and statistical significance was taken in cases of *P*-value less than 0.05.

Results

Material examinations

Deformity in all stents increased with holding force. Figure 2 illustrates the correlation between deformity and holding force in all stents. In 20-mm stents, the new stent and the PLAMAZ stent showed a similar correlation, reaching 50% deformity at approximately 8 Newtons. However, the LUMINEX stent was more significantly deformed at the same pressure, showing less than one-third the holding force of the other two stents. In 10-mm stents, the PLAMAZ stent showed a slightly higher holding force than the new stent. Both stents demonstrated about 50% deformity force at 4 Newtons.

Animal examinations

There were no premature rabbit deaths during the observation period. Multi-detector row CT demonstrated the 3-D structure of the metal frame of the implanted stents (Fig. 3a). None of the stents had metal fractures nor deformation of the main structure resulting in the preservation of the internal space within the metal structure. There was no significant difference in the diameter of the inner-frame cavity at the edges or in the middle of the stents in either group (5.70 ± 0.17 mm in the group A and 5.60 ± 0.27 mm in the group B: $P = 0.07$) (Fig. 3b).

The visual appearance of the inner cavity of the trachea by bronchofiberscope showed a significant diffidence between groups A and B. In the trachea of all five group-A (polyurethane-coated stent) rabbits, the implanted stent displayed a smooth surface covered with thin epithelium-like membranes, with mild bulging at the edge of the stents (Fig. 4a). In contrast, tracheal images for all group-B rabbits (non-coated stent) displayed thick membranes that had formed with many bulges, especially at

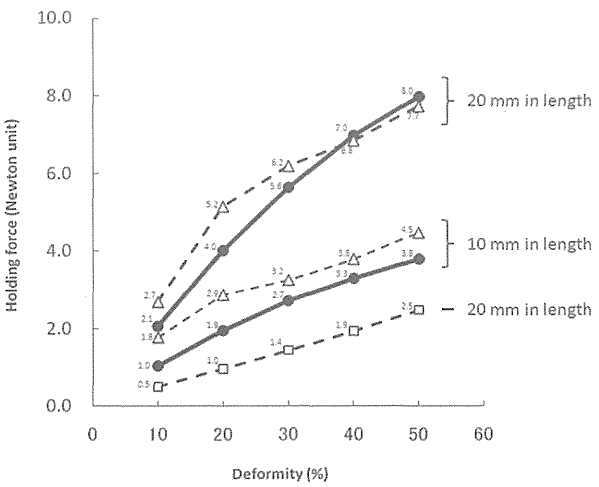


Fig. 2 Correlation between holding force and deformity. Data of holding force is labeled on the left-shoulder in each dot. The newly invented stent demonstrated similar mechanical properties to PLAMAZ metallic stents, while LUMINEX silicone stents showed significantly weaker holding force to other stents. ●, New stent; △, PLAMAZ stent; □, LUMINEX stent.

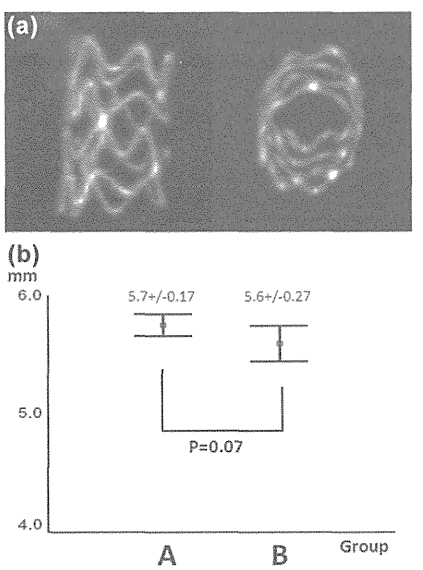


Fig. 3 Geometrical evaluation of implanted stents by multi-detector row computed tomography. (a) 3-D reconstructed image of implanted stents. No deformation was found and inner cavities were widely preserved in all stents. (b) Comparison of inner diameter of coated and non-coated stents (groups A and B, respectively). No significant difference in diameter was found in either group.

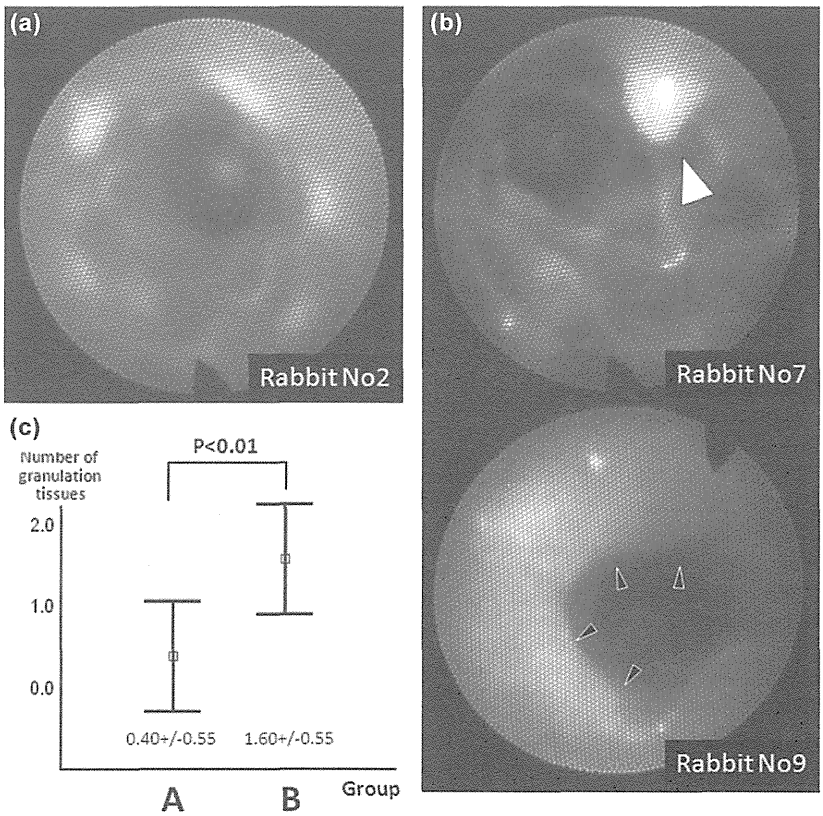


Fig. 4 Bronchofiberscopic images of coated and non-coated stents. (a) Image of implanted, coated stents. No obvious granulation tissues were found; metallic frame seems covered in tracheal membrane. (b) Image of implanted non-coated stents. Protrusion (white arrow) and significant bulging (black arrow) of granulation tissue recognized. (c) Number of granulation lesions in each group. Non-coated stents (group B) have more granulation lesions than coated stents (group A).

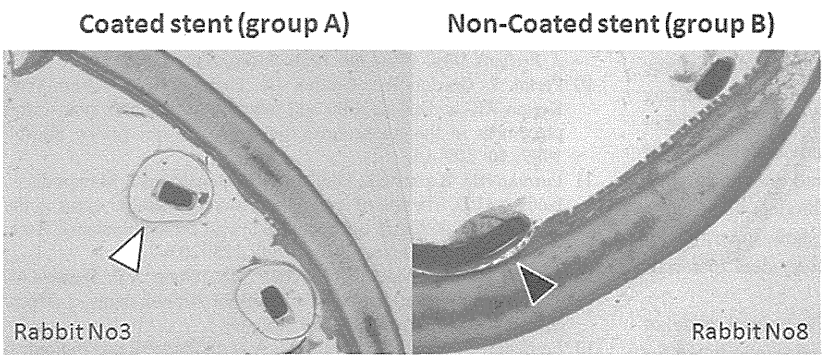


Fig. 5 Images of histological sections of implanted stents. (a) Coated stent, preserving tracheal structure (white arrow); (b) non-coated stent damage to tracheal epithelia and cartilage (black arrow).

the edges of the stents (Fig. 4b). The number of granuloma protrusions was significantly higher in group B than in group A (0.40 ± 0.55 in group A and 1.60 ± 0.55 in group B: $P < 0.01$) (Fig. 4c).

Stained sections demonstrated the histological differences between groups A and B (Fig. 5). In group A, the tracheal epithelia were less damaged, and the polyurethane structure was preserved. In group B, destruction of tracheal wall was recognized around the site of direct metal on epithelia contact, with epithelial structures destroyed.

Discussion

We invented a new metallic stent for possible treatment of TBM. Polyurethane possesses high ductility and malleability, and is ideal for coating metal because the polymer structure does not rupture. Polyurethane-covered breast implants have been widely used for a long time, with no higher risk than other materials.[5,6] Long-term, polyurethane vascular grafts demonstrate similar bioactivity to polytetrafluoroethylene grafts.[7] Polyurethane dressings were more efficient than silicone sheets on hypertrophic

scars.[8] Thus, polyurethane demonstrates low bioactivity, suggesting that a polyurethane-coated metallic stent is a reasonable choice to reduce biohistological reaction in the trachea of children.

In the materials examination, the new stent demonstrated a similar value for holding force to the PLAMAZ stent, which is a common balloon-expandable stainless steel stent. The PLAMAZ is a vascular stent designed to maintain vascular cavity during vessel stenosis, and has been applied to tracheal SI against tracheal stenosis or TBM.[9] There is only one report of physical malfunction, a case of frame deformation. Further stenoses caused by granuloma occurred as a result of mechanical stress, and histological reactions between epithelia and the metal.[10,11] These findings suggest that our new stent, like the PLAMAZ stent, has enough holding pressure to prevent the collapse of an airway for treatment of TBM or tracheal stenosis.

In the animal examination, the 3-D structure of the metal frame was preserved in all stents, which reconstructed images of the metallic frame proved. In general, the thoracic pressure is lower than the tracheal pressure in the inspiratory phase and higher in the expiratory phase. A continuous low pressure difference between the intra- and extra-airway cavities in the thorax may result in a fracture in the frame of the tracheal SI due to metal fatigue.[12] Although our results show neither fracture nor deformation of the metallic frame, there was a weak trend of difference between coated and non-coated stents suggesting unknown risk of metal fatigue with the new stent in tracheal SI. Further investigation of durability of implanted metals is needed.

Materials other than stainless steel are available for treatment of TBM. Silicone stents do not stimulate tracheal epithelia much because of low bioactivity and low foreign body reaction.[13] However, silicone stents have significantly lower holding force than metallic stents, and possess no mesh structure, reducing epithelial function in the airway.[14] In contrast, our polyurethane-coated stainless-steel stent includes both advantages of low bioactivity to the surface of the epithelium and enough holding force to maintain the cavity of the airway. No biodegradable stent is available yet, but resorbable biopolymers show superior biocompatibility to metallic stents.[15–17] Thus, more ideal materials for tracheal stenting are expected.

There are several limitations in this study. No animal model of TBM is still available while the rabbit is a good size to breed for a few weeks. The observation period may not have been long enough for long-term evaluation of metal endurance and chronic reaction to foreign bodies between stents and trachea in the animal examination. Also, long-term degradation of coating materials needs to be investigated. A future possibility could be drug-eluting stents, or biodegradable stents, which may induce less bioactivity in the trachea. Further assessment of new innovations may be required for applying treatment for TBM in daily clinics.

In conclusion, we invented a new polyurethane-coated metallic stent to reduce the histobiological reaction in the trachea to foreign bodies for treatment against TBM. The stent retains enough holding force in its metallic frame, and displays lower bioactivity to bare metallic stents. These merits will reduce complications caused by SI for treatment of airway stenoses or collapse and coated stents will represent a possible first choice for treatment of TBM in children.

Acknowledgments

None of the authors has anything to disclose.

References

1 Pillai JB, Smith J, Hasan A, Spencer D. Review of pediatric airway malacia and its management, with emphasis on stenting. *Eur. J. Cardiothorac Surg.* 2005; **27**: 35–44.
2 Gross RE. Arterial malformations which cause compression of the trachea or esophagus. *Circulation* 1955; **11**: 124–34.
3 Backer CL, Ilbawi MN, Idriss FS, DeLeon SY. Vascular anomalies causing tracheoesophageal compression. Review of experience in children. *J. Thorac. Cardiovasc. Surg.* 1989; **97**: 725–31.
4 Fraga JC, Filler RM, Forte V, Bahoric A, Smith C. Experimental trial of balloon-expandable, metallic Palmaz stent in the trachea. *Arch. Otolaryngol. Head Neck Surg.* 1997; **123**: 522–8.
5 Boyes DC, Adey CK, Bailar J *et al.* Safety of polyurethane-covered breast implants. Expert Panel on the Safety of Polyurethane-covered Breast Implants. *CMAJ* 1991; **145**: 1125–32.
6 Handel N, Gutierrez J. Long-term safety and efficacy of polyurethane foam-covered breast implants. *Aesthet. Surg. J.* 2006; **26**: 265–74.
7 Kiyama H, Imazeki T, Kurihara S, Yoneshima H. Long-term follow-up of polyurethane vascular grafts for hemoaccess bridge fistulas. *Ann. Vasc. Surg.* 2003; **17**: 516–21.
8 Wigger-Albert W, Kuhlmann M, Wilhelm D *et al.* Efficacy of a polyurethane dressing versus a soft silicone sheet on hypertrophic scars. *J. Wound Care* 2009; **18**: 208, 10-4.
9 Filler RM, Forte V, Fraga JC, Matute J. The use of expandable metallic airway stents for tracheobronchial obstruction in children. *J. Pediatr. Surg.* 1995; **30**: 1050–5; discussion 55-6.
10 Perini S, Gordon RL, Golden JA, LaBerge JM, Wilson MW, Kerlan RK Jr. Deformation and migration of Palmaz stents after placement in the tracheobronchial tree. *J. Vasc. Interv. Radiol.* 1999; **10**: 209–15.
11 Furman RH, Backer CL, Dunham ME, Donaldson J, Mavroudis C, Holinger LD. The use of balloon-expandable metallic stents in the treatment of pediatric tracheomalacia and bronchomalacia. *Arch. Otolaryngol. Head Neck Surg.* 1999; **125**: 203–7.
12 Pelton AR, Schroeder V, Mitchell MR, Gong XY, Barney M, Robertson SW. Fatigue and durability of Nitinol stents. *J. Mech. Behav. Biomed. Mater.* 2008; **1**: 153–64.
13 Furuzono T, Wang PL, Korematsu A *et al.* Physical and biological evaluations of sintered hydroxyapatite/silicone composite with covalent bonding for a percutaneous implant material. *J. Biomed. Mater. Res. B. Appl Biomater* 2003; **65**: 217–26.
14 Ernst A, Majid A, Feller-Kopman D *et al.* Airway stabilization with silicone stents for treating adult tracheobronchomalacia: a prospective observational study. *Chest* 2007; **132**: 609–16.
15 Sewall GK, Warner T, Connor NP, Hartig GK. Comparison of resorbable poly-L-lactic acid-polyglycolic acid and internal Palmaz stents for the surgical correction of severe tracheomalacia. *Ann. Otol. Rhinol. Laryngol.* 2003; **112**: 515–21.
16 Saito Y, Minami K, Kobayashi M *et al.* New tubular bioabsorbable knitted airway stent: biocompatibility and mechanical strength. *J. Thorac. Cardiovasc. Surg.* 2002; **123**: 161–7.
17 Saito Y, Minami K, Kaneda H *et al.* New tubular bioabsorbable knitted airway stent: feasibility assessment for delivery and deployment in a dog model. *Ann. Thorac. Surg.* 2004; **78**: 1438–40.

NEW RESEARCH

JCSM
Journal of Clinical Sleep Medicine

http://dx.doi.org/10.5664/jcsm.3542

A Case of Congenital Central Hypoventilation Syndrome with a Novel Mutation of the *PHOX2B* Gene Presenting as Central Sleep Apnea

Yuko Amimoto, M.D.[1]; Kenji Okada, Ph.D.[1]; Hiroshi Nakano, Ph.D.[2]; Ayako Sasaki, Ph.D.[3]; Kiyoshi Hayasaka, Ph.D.[3]; Hiroshi Odajima, Ph.D.[1]

[1]Department of Pediatrics, Fukuoka National Hospital, Fukuoka, Japan; [2]Sleep Disorders Center, Fukuoka National Hospital, Fukuoka, Japan; [3]Department of Pediatrics, Yamagata University School of Medicine, Yamagata, Japan

CASE REPORTS

Congenital central hypoventilation syndrome (CCHS) is a rare disease characterized by abnormal autonomic control of breathing resulting in hypoventilation. We report an infant girl with CCHS who presented with central sleep apnea, which was first demonstrated by polysomnography when the infant was 5 months old. She was heterozygous for the novel 590delG mutation of *PHOX2B*, which is classified as a non-polyalanine repeat mutation (NPARM). This mutation is considered to be associated with a relatively mild phenotype.

Keywords: Central sleep apnea, congenital central hypoventilation syndrome (CCHS), PHOX2B

Citation: Amimoto Y; Okada K; Nakano H; Sasaki A; Hayasaka K; Odajima H. A case of congenital central hypoventilation syndrome with a novel mutation of the *PHOX2B* gene presenting as central sleep apnea. *J Clin Sleep Med* 2014;10(3):327-329.

Congenital central hypoventilation syndrome (CCHS), which was first described by Mellins et al. in 1970, is a rare congenital disease characterized by hypoventilation.[1] Amiel et al., Sasaki el al., and Weese-Mayer et al. identified the paired-like homeobox 2B (*PHOX2B*) gene mutation in CCHS patients.[2-4] Subsequently, Weese-Mayer and colleagues identified mutations in exon 3 of the *PHOX2B* gene in all patients with the CCHS phenotype.[5] Currently, identification of a *PHOX2B* mutation is required to confirm the diagnosis of CCHS. CCHS patients characteristically demonstrate alveolar hypoventilation with diminutive tidal volumes and monotonous respiratory rates during sleep, and in severe cases, also during wakefulness.[5] Affected individuals have diffuse autonomic nervous system dysregulation (ANSD), with anatomical manifestations such as the risk of tumor development. Mcconville et al. identified two *PHOX2B* mutations (600delC, a frameshift mutation and G197D, a missense mutation) as a rare cause of non-syndromic neuroblastoma, which indicates that the underlying *PHOX2B* mutational mechanism influences the risk of tumor and suggests that the position of missense mutations may influence the resulting phenotype.[6]

We report an infant with CCHS who presented with central sleep apnea, which was first demonstrated by polysomnography (PSG) when the infant was 5 months old. She was heterozygous for the novel 590delG mutation of *PHOX2B*.

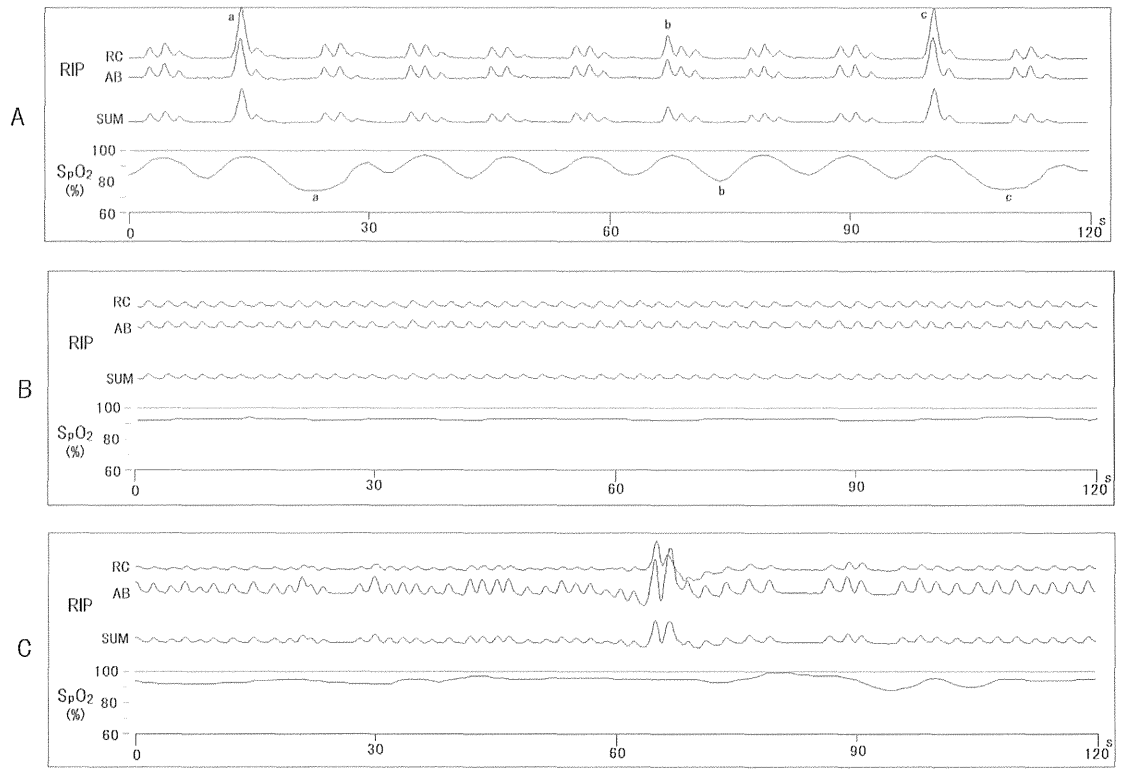
REPORT OF CASE

The patient was a 5-month-old girl delivered by Cesarean section performed for obstructed labor at 40 weeks of gestation without any other complication during pregnancy and delivery. The Apgar scores were 8 at 1 min and 9 at 5 min. She was born to healthy parents without consanguinity: a 33-year-old father and a 23-year-old mother. No relatives of either parent suffered from sleep disorders. Three of the infant's grandparents had undergone surgery under anesthesia without any problems of respiratory management. Neither the parents nor the siblings showed any signs of Hirschsprung disease, tumors of neural crest origin, or other symptoms suggestive of ANSD.

The patient was admitted to NICU because of frequent episodes of respiratory arrest for a few seconds at the onset of sleep on the day of birth. There were no rales or heart murmurs. The muscle tone was good. Venous blood gas analysis revealed a $PvCO_2$ of 32.7 mm Hg in room air. There were no abnormalities on x-ray examination of the chest/abdomen, examination of the cerebrospinal fluid, or ultrasound examination of the brain and heart. The patient was discharged from the NICU one month after birth under home oxygen therapy; supplemental oxygen by nasal cannula (2 L/m) during sleep with SpO_2 monitoring was prescribed at discharge. She was brought to the hospital in which she was born at 5 months of age with a history of frequent episodes of apnea and hypoxemia (lowest $SpO_2 \approx 70\%$) observed during sleep, and was hospitalized at the Fukuoka National Hospital for investigation of the etiology of her condition.

Initial vital signs at admission revealed an SpO_2 of 96% (room air), pulse rate of 134 beats/min, respiratory rate of 34/min, and body temperature of 36.0°C. Physical examination was unremarkable; the color and tone were good. Arterial blood gas analysis was normal (SaO_2 96.2%, PaO_2 85.2 mm Hg, $PaCO_2$ 44.0 mm Hg, HCO_3^- 25.4 mmol/L, and pH 7.379) while awake in room air. The complete blood cell count and blood chemistry profile were within normal limits. There were no abnormalities on chest x-ray, ultrasound examination of heart, abdomen or brain, electrocardiogram, laryngoscopy, electroencephalogram, brain MRI, or examination of vanillylmandelic acid (VMA)/

Figure 1—Representative traces of the respiratory pattern during sleep.



SpO_2 was measured with a toe probe. Therefore, there is a lag time between change in RIP signal and change in SpO_2 (≈ 10s). **(A)** NREM sleep. Frequent central apnea episodes are seen. Marked increase in tidal volume associated with profound desaturation was observed (a-c). **(B)** NREM slow wave sleep. Regular breathing pattern is seen. The SpO_2 is slightly reduced. **(C)** REM sleep. Irregular breathing and sporadic central apnea episodes are seen.

homovanillic acid (HVA) excretion in the urine. These results suggested the absence of any primary pulmonary, cardiac, or neuromuscular disease, or brainstem disorder.

Diagnostic PSG was performed without supplemental oxygen administration. The PSG revealed almost normal sleep architecture except for relatively frequent awakenings during night. The respiratory signals showed frequent central apnea episodes, with an apnea-hypopnea index (AHI) of 161/h. The mean apnea duration of the central apnea episodes was 5 s (range 3-24 s). The apnea events caused recurrent SpO_2 drops without associated arousal response. The lowest SpO_2 recorded was 45%. The frequency of central apnea episodes was greater during NREM sleep than during REM sleep (178/h vs. 96/h). During NREM sleep, the frequency of central apnea episodes and desaturation were the severest at sleep onset (**Figure 1A**), becoming milder as time went by. There were also stable NREM slow wave sleep periods with a regular respiratory rhythm and a slightly reduced oxygen saturation level (SpO_2 91% to 93%) (**Figure 1B**), suggesting the existence of only mild hypoventilation. The mean SpO_2 during PSG was 93.2%.

Genetic study was performed using peripheral blood cells. On amplification and sequencing of the *PHOX2B* gene, heterozygosity for a novel 590delG mutation of *PHOX2B* was detected; on this basis, the infant was diagnosed as having CCHS. Although we recommended a genetic study of the patient's family, only the patient's mother gave consent for such a study, which yielded a normal result.

We introduced noninvasive positive pressure ventilation (NIPPV) for the treatment of central sleep apnea. The initial settings for the NIPPV were: inspiratory positive airway pressure (IPAP) 8 cm H_2O, expiratory positive airway pressure (EPAP) 4 cm H_2O, respiratory rate (RR) 20 breaths/min, inspiration time 0.6 seconds. PSG under NIPPV revealed a marked decrease in the frequency of the central apnea episodes; the AHI was 10.5 and the mean SpO_2 during the PSG was 98.0%. The mean apnea duration was 7 s (range 4-12 s). The lowest SpO_2 recorded was 85%. However, three-fourths of the apnea-hypopnea episodes were classified as obstructive or mixed type, indicating inadequate NIPPV settings. Therefore, the pressure setting was increased.

DISCUSSION

We report a case of CCHS presenting as severe central sleep apnea with a novel mutation of the *PHOX2B* gene. Previous studies have reported various types of *PHOX2B* gene mutations in patients with CCHS. The present case had a novel mutation, namely, a 590delG mutation, of the *PHOX2B* gene.

It is reported that central sleep apnea is a relatively common phenomenon in normal infants.[7] However, the frequency of

central apnea events in this case was extremely high, suggestive of an unusual etiology, which was the reason for our considering genetic testing for CCHS.

PHOX2B is the disease-defining gene for CCHS. Approximately 90% of individuals with the CCHS phenotype are heterozygous for a polyalanine repeat expansion mutation (PARM), and the remaining approximately 10% of individuals with CCHS are heterozygous for a non-PARM (NPARM) (including missense, nonsense, and frameshift mutation) in the *PHOX2B* gene.[5] In this case, 590delG, a frameshift mutation (NPARM), was found in exon 3 of the *PHOX2B* gene. In contrast to the PARMs, the majority of NPARMs occur *de novo* and are associated with very severe phenotypes, including Hirschsprung disease with extensive gut involvement, need for continuous ventilatory support, and increased tumor risk.[5] Most NPARMs are considered to act in a dominant-negative and gain-of-function manner and to be associated with severe phenotypes. However, at present, our patient does not have any severe complications, except the need for ventilatory support during sleep. A few similarly located frameshift mutations (618delC, 577delG) were detected in families with milder phenotypes showing variable penetrance.[8] The 590delG mutation is expected to produce p.G197Afs*111, which may have a milder pathogenic effect than other NPARMs. We could not confirm the penetrance because the father refused to provide a specimen.

CCHS patients usually present with hypoventilation and hypoxemia. They lack both the hypercapnic ventilatory response and hypoxic ventilatory response.[9,10] In this case, while the central sleep apnea was severe, the hypoventilation seemed relatively mild, even during sleep. Although we did not perform tests for ventilatory responses, we think that the patient had a relatively preserved ventilatory response to hypercapnia or hypoxia, because she showed repetitive short desaturation-resaturation cycles, and not sustained severe desaturation during sleep. Moreover, the patient responded to severe desaturation with marked increase in the tidal volume, which also suggested preserved ventilatory responses. These observations and the presumably normal ventilatory control in the awake state, suggested by the normal $PaCO_2$, indicate that the degree of hypoventilation in this patient was considerably mild in the spectrum of CCHS. This notion was also supported by the absence of overt respiratory arrest in the first 5 months of life in the absence of any ventilatory support. We speculate that the incompletely preserved ventilatory response was the reason why this patient presented with central sleep apnea rather than severe hypoventilation.

Although positive pressure ventilation via tracheostomy is recommended during the first several years of life when brain growth and development requiring normoxia occurs,[5] we selected NIPPV for the treatment of central apnea because of the family's strong desire to avoid tracheostomy and the above-mentioned relatively preserved ventilatory responses. However, there are a few risks associated with this line of management that should be borne in mind during the follow-up period: the risk of development of mid-face hypoplasia and the uncertainty of prevention of central apnea.[5] Therefore, careful follow-up of the respiratory status and also monitoring for possible complications of NIPPV as well as emergence of neural crest tumor.[5]

This case had a novel mutation of *PHOX2B* NPARM, and suggested that the severe central sleep apnea is a phenotype of this genotype. This case experience suggests that children with severe central sleep apnea need not only neurological examination, but also genetic testing for mutations of the *PHOX2B* gene.

REFERENCES

1. Mellins RB, Balfour HH Jr, Turino GM, Winters RW. Failure of autonomic control of ventilation (Ondine's curse). *Medicine (Baltimore)* 1970;49:487-526.
2. 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.
3. Sasaki A, Kanai M, Kijima K, et al. Molecular analysis of congenital central hypoventilation syndrome. *Hum Genet* 2003;114:22-6.
4. 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.
5. Weese-Mayer DE, Berry-Kravis EM, Ceccherini I, Keens TG, Loghmanee DA, Trang H. An Official ATS Clinical Policy Statement: Congenital Central Hypoventilation Syndrome. *Am J Respir Crit Care Med* 2010;181:626-44.
6. McConville C, Reid S, Baskcomb L, Douglas J, Rahman N. PHOX2B analysis in non-syndromic neuroblastoma cases shows novel mutations and genotype-phenotype associations. *Am J Med Genet A* 2006;140:1297-301.
7. McNamara F, Sullivan CE. Sleep-disordered breathing and its effects on sleep in infants. *Sleep* 1996;19:4-12.
8. Weese-Mayer DE, Rand CM, Berry-Kravis EM, et al. Congenital central hypoventilation syndrome from past to future: model for translational and transitional autonomic medicine. *Pediatr Pulmonol* 2009;44:521-35.
9. Fleming PJ, Cade D, Bryan MH, Bryan AC. Congenital central hypoventilation and sleep state. *Pediatrcs* 1980; 66: 425-8.
10. Guilleminault C, McQuitty J, Ariagno RL, et al. Congenital central alveolar hypoventilation syndrome in six infants. *Pediatrics* 1982;70:684-94.

SUBMISSION & CORRESPONDENCE INFORMATION

Submitted for publication May, 2013
Submitted in final revised form October, 2013
Accepted for publication October, 2013

Address correspondence to: Yuko Amimoto, M.D., Department of Pediatrics, Fukuoka National Hospital, 4-39-1, Yakatabaru, Minami-ku, Fukuoka-city, Fukuoka, 811-1394, Japan; Tel: +81-92-565-5534; Fax: +81-92-566-0702; E-mail: yuyuyun0101@yahoo.co.jp

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

先天性中枢性低換気症候群の現況と展望

Congenital central hypoventilation syndrome: paradigm shifts and future prospects

早坂 清　佐々木綾子

Abstract

Congenital central hypoventilation syndrome (CCHS) is characterized by a failure of the automatic control of breathing during sleep, and is caused by the dominant *PHOX2B* mutation. *PHOX2B* encodes a highly conserved homeobox transcription factor with two short polyalanine tracts. More than 90 % of patients carry polyalanine expansion mutations (PARM) in the polyalanine tract of 20 residues and less than 10 % of the patients have missense, nonsense, or frameshift mutations (non-PARM). Approximately 25 % of the patients with PARM inherited the mutation from asymptomatic parents with somatic mosaicism or few affected parents. Molecular analysis can provide the definite diagnosis and clinically useful information. Model mouse experiments and MRI study of the patients will contribute to understanding the pathogenesis and development of new treatment strategy.

Key words: congenital central hypoventilation syndrome, PHOX2B, polyalanine, late onset central hypoventilation syndrome

はじめに

覚醒時における呼吸は，血中の炭酸ガスや酸素濃度およびpHを感知する化学受容器や肺の伸展受容器などからのシグナルが呼吸中枢で統合され，更に大脳を含む上位構造からの制御も受け調節されている．一方，睡眠時（特にnon-REM期）における呼吸は，主として化学的調節機構により制御されている（**図1**）．

先天性中枢性低換気症候群（congenital central hypoventilation syndrome: CCHS）（MIM 209880）は，呼吸中枢の化学的調節機構の先天的な異常により，主に睡眠中に，重症型では覚醒時にも低換気をきたす疾患である．有病率は，欧米の報告では，出生5万-20万人に1人と推定されている．国内の詳細なデータはないが，著者らの解析からは我が国でも少なくとも出生約10万人に1人検出されている．

病因遺伝子*PHOX2B*が解明され，本症の概要が明らかにされるとともに，*PHOX2B*遺伝子改変マウスの解析から病態が解明され始めている．本稿では，これらを踏まえて解説する．

1．臨　床

1）症　状

典型的な症例では新生児期より睡眠時に認められる中枢性の無呼吸・低換気を特徴とし，重症例では，覚醒時にも低換気が認められる．病因が*PHOX2B*変異と確認されたことから，乳児期～成人期に発症する非典型的な症例，late

Kiyoshi Hayasaka, Ayako Sasaki: Department of Pediatrics, Yamagata University School of Medicine　山形大学医学部 小児科学教室

0047-1852/14/¥60/頁/JCOPY

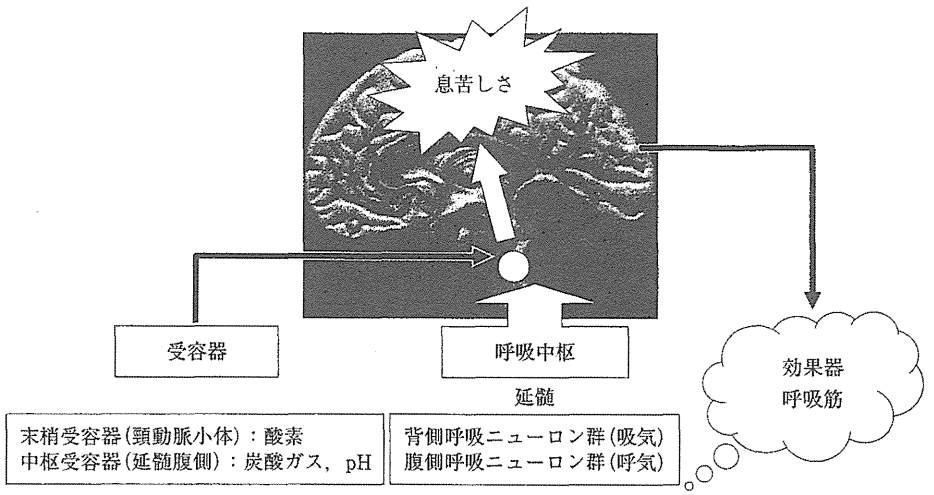


図1　呼吸の化学的調節機構

onset central hypoventilation syndrome(LO-CHS)の存在も明らかにされた．ただし，LO-CHSの約30％には*PHOX2B*変異が検出されない．CCHSでは，明らかな循環器・呼吸器疾患，筋・神経疾患，代謝性疾患，先天奇形などが認められないことが定義となっている．換気障害に気づかれず，換気障害の結果として生じた肺高血圧や心不全と診断されたり，apparent life threatening event(ALTE)を呈する症例も存在する[1]．

合併症としては，巨大結腸症(Hirschsprung病)(約20％)，神経芽細胞腫(約6％)，自律神経系の異常(心拍の呼吸性変動の低下，洞結節不全や房室ブロックなどの不整脈，便秘，食道蠕動異常，胃食道逆流症，低体温，痛覚異常，瞳孔異常，涙液分泌異常，発汗異常，体温調節障害など)があり，これらは神経堤の分化異常により説明される．加えて，低血糖や食後高血糖の報告もあり，前者は高インスリン血症，後者は自律神経の障害による血糖の調節機構の障害が考えられている[1,2]．

中枢神経系の症状として軽度の精神発達障害や視覚認知障害が報告されている．MRIでは脳幹および小脳から前脳にわたる多くの部位の構造異常が報告され，拡散強調画像では中脳，大脳脚，橋，外側延髄，小脳，上小脳脚交差や小脳脚の軸索，上下小脳脚などの髄鞘形成障害が報告されている．また，脳梁における運動，認知，会話，眼球運動調節にかかわる神経線維の減少が報告されている[3]．これらの中枢神経系の障害では，低換気による低酸素障害に基づく二次的病変を念頭に置く必要がある．

表1　睡眠時無呼吸症候群の分類

1) 中枢性睡眠時無呼吸症候群
　(1) 一次性
　　congenital central hypoventilation syndrome
　　late-onset central hypoventilation syndrome
　　Arnold-Chiari奇形
　　特発性視床下部機能不全
　(2) 二次性
　　外傷
　　梗塞
　　感染
　　腫瘍
　　頭蓋内圧亢進　など
2) 閉塞性睡眠時無呼吸症候群
　アデノイド(口蓋扁桃肥大)　など
3) 混合性睡眠時無呼吸症候群
　生理学的には閉塞性の亜型と考えられる

2) 診　断

本症の病態は，高炭酸ガス血症と低酸素血症に対する呼吸中枢の換気反応の欠如もしくは低下と考えられており，睡眠ポリソムノグラフィを用いて睡眠時の中枢性の低換気を確認する．**表1**に鑑別上重要な睡眠時無呼吸症候群の分類

を示す．呼吸機能検査では，標準的な検査に加えて，炭酸ガスに対する換気応答試験を行い診断される．新生児における呼吸機能検査には熟練を要し，近年，病因の解明に伴い，*PHOX2B*遺伝子解析により診断される症例が増加している．

3）治　療

安全な呼吸管理が求められる．欧米における治療指針では，生後数年は気管切開のもと陽圧式人工呼吸器を用いて管理する．その後にバイパップ(bilevel positive airway pressure: BiPAP)，胸郭外陰圧式人工呼吸器(continuous negative extrathoracic pressure ventilation: CNEP)や横隔膜ペーシングなどを用いて管理する[1]．低換気が睡眠時のみに認められる症例では睡眠時のみに，覚醒時にも認められる症例では覚醒時にも呼吸を管理する．国内では，新生児期からBiPAPを用いることが少なくなく，外見を気にする保護者の日本人気質による影響が考えられる．しかし，安全面および顔面骨の変形をきたすことから推奨されない．横隔膜ペーシングは，患者の行動範囲を広げ生活の質を高めるが，国内では一般的でなく，欧米で施術された1例のみ存在する．

4）予　後

フランスからの報告では，死亡率は38％，死亡例の中央値は3カ月であり，主な死因は肺性心，肺炎，窒息である[4]．長谷川らによる国内の37症例の報告では，7例(19％)が死亡しており，22例では診断後の障害は進行せず，8例には障害を認めていない[5]．今後，診断法および呼吸管理法の向上により予後の改善が期待される．

2．病　態

1）病因遺伝子*PHOX2B*と変異

*PHOX2B*と病因が特定される前には，神経堤の分化誘導にかかわる多くの遺伝子，*RET*，*GDNF*，*EDN3*，*BDNF*，*HASH1*，*PHOX2A*，*GFRA1*，*BMP2*，*ECE1*などの変異が報告されていた．しかし，いずれも少数であり，伸長変異検出法の改善により*PHOX2B*変異も検出されたことから，これらの変異の有無にかかわらず，*PHOX2B*変異がCCHSの一次的病因であることが明らかにされた[1,6,7]．

PHOX2B(MIM 603851)は，染色体4p12に位置し，約5Kbの大きさで，3つのエクソンから構成される．この遺伝子は，9個と20個のアラニンからなる2つのポリアラニン鎖を各1個，homeoboxを1個有する転写調節因子をコードしている(図2)．アミノ酸配列は，チンパンジー，ラット，マウスまで100％一致しており，哺乳動物における重要な役割を示唆している．後述するように，PHOX2Bは，呼吸中枢の形成および自律神経系の分化・誘導に重要な役割を有している．

症例は変異のヘテロ接合体であり，優性遺伝形式をとる．90％以上の症例には，20ポリアラニン鎖における4–13アラニンの伸長変異(24–33ポリアラニン変異)(polyalanine repeat expansion mutation: PARM)が検出され，約10％には，ミスセンス，ナンセンス，フレームシフト変異などの非アラニン伸長変異(nonpolyalanine repeat expansion mutation: NPARM)が検出される(図2)[1,6,7]．

伸長変異の遺伝について，ほとんどの変異は*de novo*の変異と考えられてきた．しかし，感度の高いフラグメント解析を用いて両親のゲノムDNAを解析すると，伸長変異の約25％はモザイクの親や遺伝子変異を有する無症状の親(不完全浸透を示す24もしくは25ポリアラニン変異を有する)およびLO–CHSに罹患している親からの遺伝であることが明らかにされた[8,9]．フラグメント解析は，モザイク率2％まで検出可能な鋭敏な方法であり，本解析により次子への遺伝の危険性が判明すると考えられたが，最近，性腺モザイクの一報告があり，末梢血のゲノムDNAを用いた方法では，検出できない性腺モザイクの存在が明らかにされた[10]．

伸長変異の由来について，アラニンのコドンはGCNであり，ポリアラニン配列はGC塩基の多い反復配列で，健康な人に短縮配列が多型として検出されることから，減数分裂時における不等交差により生成されると考えられてきた．

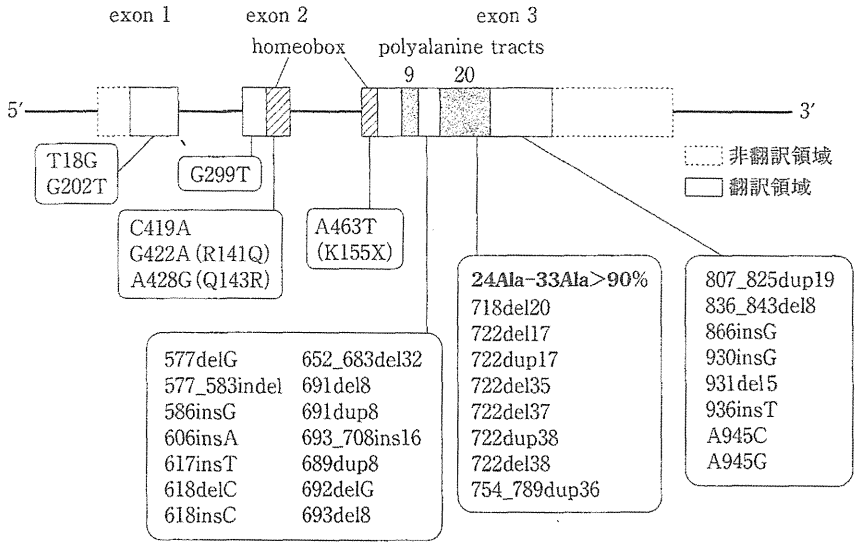


図2 *PHOX2B* の構造と変異

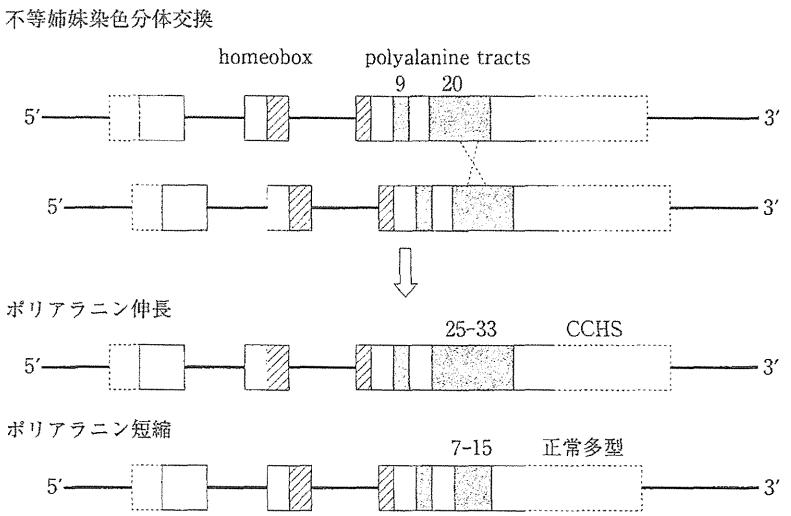


図3 *PHOX2B* のポリアラニン伸長・短縮機構

しかし，著者らの解析から，*de novo* の変異は，父親の精子形成時における不等姉妹染色体分体交換により生成することが明らかにされた(図3)[11]．一方，変異の一部には姉妹染色体分体間の組換えでは説明できない変異が存在する．また，伸長アリルのモザイク例では，正常アリルと伸長アリルのみが検出され，短縮アリルは検出されず，不等姉妹染色体分体交換による生成機構では説明されない．ポリアラニン配列はGC塩基の多い三塩基の反復配列であることから二次構造をとりやすく，一部の症例やモザイク例においては，DNAの複製や修復時における複製フォークの停止そして異なる部位からの再開という生成機構が推定される[12]．

2) 遺伝子型と臨床型について

25ポリアラニン変異および一部の非アラニン伸長変異(NPARM)では，不完全浸透を示し，変異を有していても必ずしも発症しない．また，新生児期以降に発症する(LO-CHS)症例，呼吸器感染症罹患時や麻酔時に低換気が顕在化する

症例も存在する[1]．合併症に関しては，巨大結腸症の合併は認めず，他の自律神経障害もほとんど認めない．最近，24ポリアラニン変異のホモ接合体およびヘテロ接合体の症例が検出されており，25ポリアラニン変異と同様にLO-CHSの発症や不完全浸透を示すことが推測される．26以上のポリアラニン変異では，完全浸透を示し，すべて新生児期に発症する．また，巨大結腸症の合併頻度が高く，巨大結腸症の合併を認めなくとも，慢性の便秘を訴える症例が多く，胃食道逆流症を認める症例も存在する．一方，断端吻合術を施行した巨大結腸症においても，残存する腸管の神経叢の発達が悪い症例では術後も通過障害を認める．26ポリアラニン変異では25％，27ポリアラニン変異では67％の症例に，不整脈のためにペースメーカーの装着を要したという報告もある．伸長の長さに比例し，他の自律神経系の異常を合併する頻度が高い．また，29以上のポリアラニン変異では，神経節細胞芽腫，神経節細胞腫の報告があり注意を要する．

ミスセンス変異，ナンセンス変異やフレームシフト変異などのほとんどの非アラニン伸長変異(NPARM)を有する症例では，26以上のポリアラニン変異を有する症例と同様に重症であることが多く，不整脈，巨大結腸症や神経芽細胞腫の合併も多く，心電図モニターや定期的な神経芽細胞腫のマーカー測定などによる経過観察が重要である．

3) モデルマウスおよび *in vitro* における発現実験

a. *Phox2b* ノックアウトマウス

Phox2b ノックアウトマウスのホモ接合体では，末梢化学受容器から呼吸の調節にかかわる中枢の形成が障害され，胎仔期に死亡する[13]．ヘテロ接合体では，新生仔期に高炭酸ガスや低酸素に対する換気応答の低下を一過性に認めるが，生後10日までには消失し成長する．CCHSとしての症状を認めないことから，CCHSに検出される *PHOX2B* 変異はloss-of-functionとしてではなく，gain-of-functionとして作用することを示唆している．

b. Constitutive $Phox2b^{27Ala/+}$ マウス

Phox2b アリルに27ポリアラニン変異をノックインしたマウスのヘテロ接合体では，生後間もなくからチアノーゼを呈し数時間で死亡する[14]．出生後の呼吸は，個体差が大きく，喘ぎ呼吸，無呼吸の混じる不安定な呼吸，浅く緩徐な呼吸など様々なパターンを示し，長時間の無呼吸が高頻度に認められる．炭酸ガスに対する換気応答を認めずCCHS類似の症状を呈する．胎生12.5日には，pre-Bötzinger複合体の機能は認められるが，retrotrapezoid nucleus(RTN)/parafacial respiratory group(pFRG)における $Phox2b^{+}$ ニューロンの脱落が認められる(図4)．後期胎仔においてはRTN/pFRGが呼吸リズムの形成にも関与し，$Phox2b^{27Ala/+}$ マウスの不安定な呼吸は，化学刺激による換気応答の障害と呼吸リズム形成の障害によると考えられる．

c. Conditional $Phox2b^{27Ala/+}$ ($=Egr2^{cre/+}$ $Phox2b^{27Alacki/+}$) マウス

RTNに限局した27ポリアラニン変異導入マウスでは，RTNが形成されず，炭酸ガスに対する換気応答が認められない[15]．しかし，pCO_2 は正常に保たれ成長する．酸素に対する換気応答が認められることから，末梢化学受容器による調節機構が作動していることが考えられる．炭酸ガスに対する換気応答は，生後3週間では正常の約20％，生後4カ月では約40％と改善してくることから，頸動脈小体や脳の他の化学受容器(セロトニンニューロンやグリア細胞など)による代償機構の作動が推察される．constitutive $Phox2b^{27Ala/+}$ マウスは，新生仔期に死亡することから，Phox2bは生命維持に重要な網様体などの形成にも重要な役割を果たしていることが推測される．

d. Constitutive $Phox2b^{del5/+}$ および $Phox2b^{del8/+}$ マウス

NPARM変異を有する症例では，巨大結腸症や神経芽細胞腫を合併する頻度が高い．NPARM変異のモデルである $Phox2b^{del5/+}$ および $Phox2b^{del8/+}$ マウスは，生後自発呼吸は認めず，数時間で死に至る[16]．RTN/pFRGニューロンの欠失に加えて，Phox2b依存性の顔面神経運動

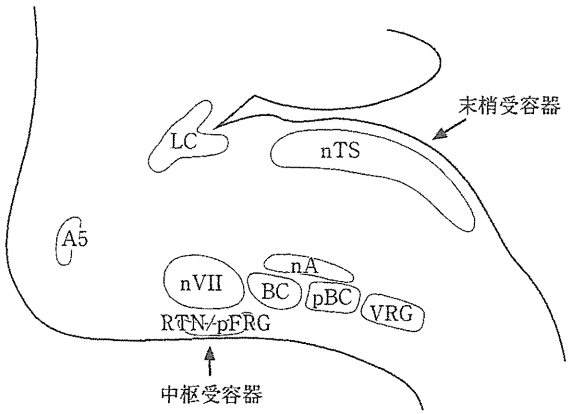


図4　*Phox2b*$^{27Ala/+}$マウス脳幹における*Phox2b*$^{+}$ニューロンの脱落

Phox2b$^{27Ala/+}$マウスでは，RTN/pFRGにおける*Phox2b*$^{+}$ニューロンの脱落が認められる．

RTN/pFRG: retrotrapezoid nucleus/parafacial respiratory group, BC: Bötzinger complex, pBC: pre-Bötzinger complex, VRG: ventral respiratory group, nA: nucleus ambiguous, nVII: nucleus facialis, nTS: nucleus of the soiltary tract, LC: locus coeruleus.

(文献[14]より改変)

核と迷走神経背側運動核の低形成～欠失も認められ，腸管の自律神経節に関しても低形成～欠失が認められる．胎仔における腸管および交感神経節の前駆細胞では，Sox10の発現が持続し，増殖が抑制され，グリア細胞系への分化が促進している．野生型Phox2bによるdopamine β-hydroxylaseに対する転写促進作用をdominant-negativeに抑制し，Sox10に対する転写抑制作用を打ち消し，促進的に作用する．Phox2bによるSox10の発現調節機構は，自律神経節の正常発達に重要な役割を果たしていると考える．

4) PHOX2Bの発現と機能

PHOX2Bは，神経細胞の分化誘導に重要な役割を果たしている転写因子であり，呼吸，循環，消化管の調節にかかわる自律神経の中枢の形成に関与している．呼吸とのかかわりでは，末梢および中枢の化学受容器からのシグナルの伝達および統合に関与する一連のニューロンに発現しており，これらのニューロンには頸動脈小体，化学受容器からの求心路，孤束核から延髄腹外側への投射路，RTNに位置する中枢化学受容器も含まれる．*Phox2b*遺伝子改変マウスの解析から，RTN/pFRGの*Phox2b*$^{+}$ニューロンは，グルタミン酸作動性でCO_2感受性を示し，中枢化学受容器として重要な役割を果たしていることが明らかにされた．このニューロンは，少なくとも齧歯類の新生仔では，呼吸リズムジェネレーターにも関与していることが明らかにされた．

3. 将来への展望

1) 診　　断

新生児における呼吸機能検査には熟練を要し，小児を対象とした簡便な方法の開発が求められる．

鑑別に関して，**表1**に示す疾患群が挙げられる．*PHOX2B*遺伝子の解析は比較的容易であり，遺伝子型と表現型の関係を更に明らかにする必要がある．一方，病因不明な疾患群も存在し，特にLO-CHSでは約30％の症例で変異が検出されず，新たな病因の解明が待たれる．

2) 病　　態

モデルマウスの解析から病態に関して多くの情報が得られているが，致死的であること，サイズが小さいことから生理学的な解析が困難で

ある．近年，MRIを用いたCCHSの脳の構造解析および機能的MRIを用いた呼吸時の脳の活動部位の解析が進んでいる[3]．症例の解析においては，低酸素による二次的な障害を除くことが重要であり，今後，幼少時から十分な呼吸管理が施行されている症例を対象として，遺伝子型のデータとともに解析することにより病態の解明が進展するものと考える．成長に伴う変化の解析やプロゲステロンなど新たな治療法の開発においても有用な情報を提供するものと考える．なお，pre-Bötzinger複合体は呼吸リズムジェネレーターとして知られているが，最近，この領域のアストロサイトがニューロンとともにリズム形成に重要な役割を果たしていることが明らかにされた[17]．

3）治　　療

国内では気管切開下の呼吸管理は好まれない状況であるが，安全な管理法の周知と非侵襲的人工呼吸の安全な運用およびモニターの開発が期待される．横隔膜ペーシングに関しては，国内における施術および普及が期待される．

新たな治療戦略として，二つの可能性が検討されている．一つは，分子シャペロン療法である．*in vitro*の発現実験で，伸長変異タンパクはfoldingが障害され，細胞質で凝集し核内への移行が妨げられ，転写因子としての作用が低下する．foldingの改善を目的に，熱ショックタンパク質を誘導するgeldanamycinなどの添加により凝集体の形成や転写活性の低下が緩和された[18]．他の神経疾患に対する治療法の研究をヒントに，*in vitro*の発現実験で17-allylamino-17-demethoxygeldamycin(17-AAG)やターメリックの主成分であるcurcuminが伸長変異*PHOX2B*の核内移行を促進し，転写活性を高め，凝集変異タンパクの除去を促進することが確認された[19]．ただし，dominant-negativeに作用する変異では，効果は期待できない．また，CCHSのモデルconstitutive $Phox2b^{27Ala/+}$マウスでは，病理学的にRTN/pFRGにおける*Phox2b*発現陽性のグルタミン作動性ニューロンの減少が確認されており，出生後に施行する分子シャペロン療法の効果は疑問視される[16]．

二つめは，プロゲステロン療法である．契機は，19歳のCCHS女性(25アラニン伸長変異)が経口避妊薬の服用により，炭酸ガスに対する呼吸応答が出現したことによる．その後，30歳CCHS女性(26アラニン伸長変異)において，desogestrel服用半年後に，炭酸ガスに対する換気応答が確認された[20]．プロゲステロンや誘導体は，肥満による低換気に有効な治療薬として使用されており，閉経期女性に対するホルモン補充療法により$PaCO_2$が低下することも報告されている[21]．プロゲステロンはラット新生仔の無呼吸頻度を減少させ，低酸素への換気応答も改善することが知られている．ただし，女性ホルモンであり，男性患者への応用の問題，効果の持続性，推定される機序など，実用に向けて解決すべき様々な問題を含んでいる．

追　加　なお，本疾患は，ゲルマン民族の民話に基づき，'オンディーヌの呪い'と呼ばれてきたが，同病名は患者に苦痛を与えており，先天性中枢性低換気症候群(CCHS)という正式名称の普及を願う．

■文　　献

1) Weese-Mayer DE, 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.
2) Gelwane G, et al: Intermittent hyperglycemia due to autonomic nervous system dysfunction: a new feature in patients with congenital central hypoventilation syndrome. J Pediatr **162**: 171-176, 2013.
3) Patwari PP, et al: Congenital central hypoventilation syndrome and the PHOX2B gene: a model of respiratory and autonomic dysregulation. Respir Physiol Neurobiol **173**: 322-335, 2010.
4) Trang H, et al: Short-term blood pressure and heart rate variability in congenital central hypoventilation syndrome(Ondine's curse). Clin Sci(Lond) **108**: 225-230, 2005.

5) Hasegawa H, et al: Epidemiologic survey of patients with congenital central hypoventilation syndrome in Japan. Pediatr Int **54**: 123-126, 2012.
6) Amiel J, 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.
7) Sasaki A, et al: Molecular analysis of congenital central hypoventilation syndrome. Hum Genet **114**: 22-26, 2003.
8) Bachetti T, et al: 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.
9) Meguro T, et al: Inheritance of polyalanine expansion mutation of PHOX2B in congenital central hypoventilation syndrome. J Hum Genet **57**: 335-337, 2012.
10) Rand CM, et al: Germline mosaicism of PHOX2B mutation accounts for familial recurrence of congenital central hypoventilation syndrome(CCHS). Am J Med Genet A **158A**: 2297-2301, 2012.
11) Arai H, 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.
12) Mirkin SM: Expandable DNA repeats and human disease. Nature **447**: 932-940, 2007.
13) Dauger S, et al: Phox2b controls the development of peripheral chemoreceptors and afferent visceral pathways. Development **130**: 6635-6642, 2003.
14) Dubreuil V, et al: A human mutation in Phox2b causes lack of CO_2 chemosensitivity, fatal central apnea, and specific loss of parafacial neurons. Proc Natl Acad Sci USA **105**: 1067-1072, 2008.
15) Ramanantsoa N, et al: Breathing without CO_2 chemosensitivity in conditional Phox2b mutants. J Neurosci **31**: 12880-12888, 2011.
16) Nagashimada M, et al: Autonomic neurocristopathy-associated mutations in PHOX2B dysregulate Sox10 expression. J Clin Invest **122**: 3145-3158, 2012.
17) Okada Y, et al: Preinspiratory calcium rise in putative pre-Bötzinger complex astrocytes. J Physiol **590**(Pt 19): 4933-4944, 2012.
18) Trochet D, et al: Molecular consequences of PHOX2B missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. Hum Mol Genet **14**: 3697-3708, 2005.
19) Di Zanni E, et al: In vitro drug treatments reduce the deleterious effects of aggregates containing polyAla expanded PHOX2B proteins. Neurobiol Dis **45**: 508-518, 2012.
20) Straus C, et al: Chemosensitivity recovery in Ondine's curse syndrome under treatment with desogestrel. Respir Physiol Neurobiol **171**: 171-174, 2010.
21) Joseph V, et al: Sex, hormones, and stress: how they impact development and function of the carotid bodies and related reflexes. Respir Physiol Neurobiol **185**: 75-86, 2013.