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4　呼吸管理法の定期的な見直し

Key point

・成長に伴う生理的変化である 1 回換気量の増加，呼吸数の減少などが起きないため，定期的に換気状態を評価し呼吸管理法を見直す必要がある．
・呼吸中枢障害についても定期的に評価する．呼吸中枢障害が進行し，睡眠時の低換気が重症化することや，乳児期には明らかでなかった覚醒時低換気が幼児期以降に顕在化することがある．
・定期的な呼吸管理の見直し，成長段階に合わせた呼吸管理への変更には，専門施設との連携が望ましい．

【呼吸状態の定期的な評価】

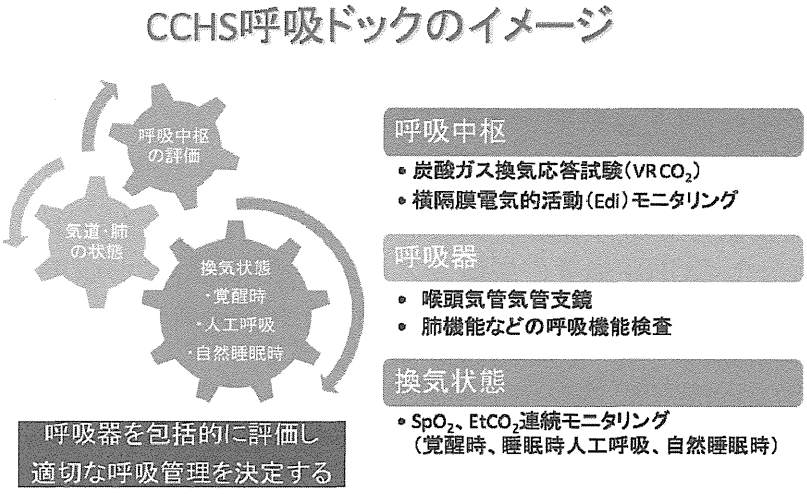
CCHS では睡眠時の人工呼吸では呼吸器に完全に同調することが多いため，成長に合わせて呼吸器の設定を変更する必要がある．乳児期の設定を続けると，圧力が不足し一回換気量の低下が起きることや，年齢相応より早い呼吸数で換気することになる．成長段階に合わせた呼吸状態になるように調整する．設定を変更する際には，再度 SpO_2，$EtCO_2$ 等の連続モニタリングを行う．

呼吸中枢障害についても定期的な評価を行うことが望ましい．定期的に連続モニタリングを行うと，睡眠時の低換気が重症化している症例もあり，出生後の低換気の蓄積により呼吸中枢障害が進行している可能性がある．また，幼児期になりテレビ，絵本を読むなど意識を集中するようになると，その時点で初めて覚醒時の低換気に気が付く症例がある．覚醒時の低換気は予後に影響するためその有無を慎重に見極めることが重要である．さらに幼児期後半に入ると，活動性が高まり運動量が増加する．運動による炭酸ガス負荷に対しても呼吸賦活は起きないと考えられるが，現時点ではその運動時の換気状態については詳細が明らかになっていない．

成長に伴う人工呼吸器設定の変更と同様に，呼吸管理法の検討を行う．気管切開からの呼吸管理を行っていた症例をいつ鼻マスク等からの呼吸管理に変更するかということについては，定まった見解はないが，考慮する一つのタイミングは就学時であると考えられる．このころには，発達が進んでいれば，人工呼吸器をつける必要性を理解しマスクの装着が容易になり，さらにマスク換気による顔面骨変形は起きにくくなっている．しかし，覚醒時の低換気がある症例では日中のマスク換気が困難であるため気管切開の継続が望ましい場合や，マスク換気の呼吸管理の不安定さからあえて気管切開を継続するという場合もある．個別性の高い問題であるため，初めに呼吸管理法を決定する時同様，保護者とそしてこの時期には児とも十分に話し合い決定すべきである．

【専門施設との連携】

このような呼吸管理の調整は，漏れのないように一定のプロトコルに基づいて行われることが望ましい．海外では以前より検査間隔，内容など一定のプロトコルに基づいて行われている施設がある．国内でも，その動きが始まっており，本ガイドライン作成時点では東京女子医科大学東医療センター新生児科を中心に，呼吸器を包括的に評価し呼吸管理を決定するプログラムである CCHS 呼吸ドックが行われている．これまでに国内症例の約 20%が何らかの形で検査を受けている．CCHS 呼吸ドックを行ったことで初めて明らかになる気道病変や覚醒維持低換気がある．その結果，呼吸管理が変更となる症例も少なくない．専門施設と連携し専門的な評価と平時の管理を分担することで，CCHS 診療の質の向上，自施設の負担軽減，複数の評価者が入ることで見逃しが減ることなどの効果も期待できるため，積極的な連携が望ましいと考えられる．



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C　薬物療法

Key point

・CCHS の呼吸不全に対する薬物療法では未だ有効なものはない.

CCHS の呼吸を安定させる呼吸賦活薬などの薬物治療は現在ない[1]. 経口避妊薬のプロゲステロンを内服した CCHS 患者の炭酸ガスに対する換気応答が改善した症例報告[2]により薬物療法として期待され, Straus 等により CCHS 女性患者 desogestrel を 112 日間投与の臨床研究が行われたが, 未だ有効性は証明されていない[3].

また, インビトロの研究では腫瘍の治療薬である 17-AAG および curcumin が *PHOX2B* の変異遺伝子のクリアランスを推進し, ポリアラニンの活動性を回復する働きが証明されており[4], 今後の検討が期待される.

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第5章　予後

A　発達予後

Key point

・気管切開の上，適切な人工呼吸管理を行うと発達予後は概ね良好である．

【発達予後】

CCHSにおける発達に関しては，低酸素による影響を除外できず，疾患自体による障害の評価は困難であった．しかし，病因遺伝子が判明したことから，早期診断・早期治療が可能となり，新生児期から適切に管理された20症例で検討が行われ，全検査IQ 84.9±23.6（平均±SD）と学童期における軽度の認知機能の低下が報告された[1]．更に，2015年，乳幼児31症例（平均月齢　25.0±8.5か月，6-40か月）が解析され，Bayley発達検査では，心的尺度83.35±24.75，運動尺度73.33±20.48と有意な低下が確認されたことから，疾患自体に基づく中枢神経系の障害が推定される[2]．心的尺度および運動尺度の低下は，重症な息とめ発作，長時間の洞停止および24時間の呼吸管理との関連，運動尺度の低下はけいれんの合併との関連が確認された[2]．

国内の報告では，苛原らは学齢期以上の17例に後方視的に調査を行い，気管切開症例は正常から重度発達遅滞まで認めたが，気管切開の時期が遅いほど遅滞が重篤である傾向を認め，非侵襲的人工呼吸管理を行った症例には全例境界域～中等度の発達遅滞を認めたと報告している[3]．また，Shimokazeらは25PARMの19例について調査し，8例（42%）に精神運動発達遅延を認めた[4]．25PARMでは発症時期が新生児期以降であったり，症状が非典型的であったりしたことから，診断が遅れ，適切な治療が行われずに中枢神経系が障害を受けたことが考えられた．

予後の改善のためには，新生児期の呼吸障害の鑑別疾患としてCCHSを念頭に置き，速やかな診断のもと，気管切開を行い，適切に呼吸管理を行うことが発達予後の改善をもたらすものと考える．

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B　生命予後

Key point

・適切な管理が行われれば，生命予後は良好である．

【生命予後】

安全で確実な呼吸管理と不整脈等の管理が行われれば，生命予後は良好である[1)]．

(文献)

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ORIGINAL ARTICLE

Genotype–phenotype relationship in Japanese patients with congenital central hypoventilation syndrome

Tomoyuki Shimokaze[1], Ayako Sasaki[1], Toru Meguro[1], Hisaya Hasegawa[2], Yuka Hiraku[3], Tetsushi Yoshikawa[4], Yumiko Kishikawa[1] and Kiyoshi Hayasaka[1,5]

Examine the genotype–phenotype relationship in Japanese congenital central hypoventilation syndrome (CCHS) patients and estimate the incidence of CCHS in Japan. Subjects were 92 Japanese patients with *PHOX2B* mutations; 19 cases carried 25 polyalanine repeat expansion mutations (PARMs); 67 cases carried 26 or more PARMs; and 6 had non-PARMs (NPARMs). We collected clinical data in all patients and estimated the development or intelligent quotients only in the patients carrying 25 PARM. The estimated incidence of CCHS was greater than one case per 148 000 births. Polyhydramnios was observed in three cases. Twelve infants exhibited depressed respiration at birth. In 19 cases carrying 25 PARM, the male-to-female ratio was ~3, no cases had Hirschsprung disease; 7 cases (37%) developed hypoventilation after the neonatal period, and 8 cases (42%) had mental retardation. In other 73 cases carrying 26 or more PARMs or NPARMs, male-to-female ratio was equal; patients frequently complicated with Hirschsprung disease and constipation, and all patients presented with hypoventilation in the neonatal period. Clinical symptoms were severe in most patients carrying long PARMs and NPARMs. In 25 PARM, additional genetic and/or epigenetic factors were required for CCHS development and male sex is likely a predisposing factor. The patients carrying 25 PARM frequently had mental retardation likely because they were not able to receive appropriate ventilation support following a definitive diagnosis owing to subtle and or irregular hypoventilation. Molecular diagnosis provides a definitive diagnosis and enables to receive appropriate ventilator support.
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INTRODUCTION

Congenital central hypoventilation syndrome (CCHS; OMIM209880) is a disorder that is characterized by a failure of the automatic control of breathing. Hypoventilation develops mainly during sleep, but can also be present during both wakefulness and sleep in severe cases.[1,2] CCHS is a neurocristopathies (a failure of the migration or differentiation of neural crest-derived precursor cells) and is frequently accompanied by Hirschsprung disease, neuroblastoma and dysregulation of the autonomic nervous system.[2–13] A report from France described an estimated incidence of CCHS of one per 200 000 live births.[14]

CCHS is caused by a mutation in *PHOX2B*, which is essential for the development of the respiratory center and for the differentiation and induction of the autonomic nervous system.[15–18] The *PHOX2B* gene is mapped on chromosome 4p12 and encodes a highly conserved homeobox transcription factor of 314 amino acids with two short and stable polyalanine repeats of 9 and 20 residues.[19] Over 90% of patients with CCHS are heterozygous for polyalanine repeat expansion mutations (PARMs) in *PHOX2B* that can range from 24 to 33 alanines, and remaining 10% of patients have heterozygous non-PARMs (NPARMs) that include missense, nonsense and frameshift mutations in *PHOX2B*.[20] Approximately 25% of the PARMs is inherited from the parents with somatic mosaicism or constitutive mutation, and the rest of ~75% is *de novo* during spermatogenesis.[21–23]

Disease severity in the patients carrying PARMs increases with increasing expansion of the alanine repeats.[20,24,25] The patients carrying long PARMs or most NPARMs present with severe phenotype in neonatal period.[26] In contrast, the individuals carrying 25 PARM or 24 PARM exhibit hypoventilation during the neonatal period or after the neonatal period and occasionally have no symptoms.[2,20]

Our facilities have provided a molecular diagnostic service for the majority of CCHS patients in Japan. Herein, we report an estimate of the incidence of CCHS in Japan and an analysis of the genotype–phenotype relationship.

METHODS

Subjects

We analyzed the genotype–phenotype relationship of a total of 92 CCHS patients (male-to-female ratio, 49:43) who were diagnosed in the past 12 years including 11 cases previously reported.[16,27–29] The median gestational age (GA)

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Table 1 Overview of *PHOX2B* coding sequence status and clinical features

			Apgar score[a]		*Age at presentation of CH*				
PHOX2B status	*Number of cases*	*Male: female*	*1 min*	*5 min*	*Neonatal period*	*After neonatal period*	*Constipation without HSCR*	*HSCR*	*Ventilation support during wakefulness*
25 PARM	19	14:5	9 (8–9)	10 (9–10)	12	7	0	1	0
26 PARM	25	12:13	7 (5–8)	8 (7–9)	25	0	2	7	1
27 PARM	31	16:15	8 (6–9)	9 (9–10)	31	0	10	13	1
>30 PARM	11	5:6	6 (6–7)	8 (7–9)	11	0	3	7	0
NPARM	6	2:4	8 (6–9)	9 (6–10)	6	0	4	4	4

Abbreviations: CH, central hypoventilation; HSCR: Hirschsprung disease; NPARM, non-PARM; PARM, polyalanine repeat expansion mutations.
[a]Values are expressed as median (IQR). Differences in Apgar score among the five *PHOX2B* groups were significant at 1 min ($P<0.01$) and 5 min ($P<0.05$), respectively.

was 39 weeks (interquartile ranges: IQR 38–40 weeks, these data were available in 81 cases), and the median birth weight was 2857 g (IQR 2602–3104 g, these data were available in 78 cases). Six cases were born at preterm delivery; one case was delivered at 33 weeks and five cases were delivered at 35–36 weeks of GA. Six cases were born post-term at 42 weeks of GA. One infant was heavy for date, and nine infants were light for date (data from 73 cases).

This study was approved by the institutional review board of Yamagata University School of Medicine, and written informed consents were obtained from the parents of all infants. The diagnoses followed the standard of the statement of the American Thoracic Society on CCHS.[26] We collected clinical data including information about complications when the blood samples were received. In the patients with 25 PARMs, the developmental quotients or intelligence quotients were assessed using Enjoji's analytical development test,[30] the revised version of the Kyoto Scale of psychological development (*K*-test),[31] or the Wechsler Preschool and Primary Scale of Intelligence.[32] The intelligence quotients were assessed with the Wechsler Intelligence Scale for Children-Third Edition.[33] The frequency of CCHS from 2008 to 2013 years was estimated comparing to the numbers of births each year in Japan, which were reported by the Ministry of Health and Labor and Welfare of Japan.

Molecular analysis

We extracted genomic DNA from peripheral blood using a standard method. The entire coding region and intron–exon boundaries of *PHOX2B* were sequenced after polymerase chain reaction amplification as described previously.[16,18,27,34] The sequence reactions were analyzed on an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA).[16,34]

Statistics

Clinical data are expressed as median and IQR or range. The Pearson χ^2-test was used to compare the proportions of male infants in the patients carrying 25 PARM and the patients carrying other genotypes. Differences between the two groups were compared with the Mann–Whitney *U*-test. The Kruskal–Wallis test was used for comparison of more than two groups. Spearman's correlation coefficients were used to identify the relationships within and between the different outcome measures. A *P*-value of <0.05 was considered to be statistically significant. The statistical analyses were performed using SPSS software, version 22 (IBM, Armonk, NY, USA).

RESULTS

A total of 92 (including with two pairs of siblings) CCHS patients were studied. Molecular analyses of the *PHOX2B* gene revealed that 86 cases had PARMs and six cases had six different NPARMs including the previously reported c.590delG and c.866InsG mutations[16,26] and the novel c.609_616del8, c.678_693dup16, c.733_762dup30 and c.941_945del5 mutations (Table 1). The patient carrying 24 PARM was not found probably owing to low gene frequency and/or low penetrance.

The minimum estimated incidence of CCHS in Japan was one per 148 000 births on average for the years of 2008–2013 (range 1 per 119 000–206 000 births).

Polyhydramnios was observed in three cases (25 PARM, 26 PARM and c.678_693dup16). The median Apgar score at one min was 8.0 (IQR 6.0–9.0, data were available in 69 cases) and at 5 min the median was 9.0 (IQR 7.5–9.5, data were available in 63 cases). Twelve infants (three with 25 PARM, five with 26 PARM, two with 27 PARM, one with 32 PARM and one with NPARM) were found to be depressed at birth (defined by an Apgar score below 4 at 1 min and/or 6 at 5 min). The correlation coefficients (r) between the alanine repeat expansions and the Apgar score were -0.32 ($P<0.01$) at 1 min, and -0.10 ($P=0.44$) at 5 min, respectively.

Among the patients with 25 PARM, the male:female ratio was 14:5; i.e., approximately threefold more males than females were affected (Table 1). In contrast, there were no gender differences in the patients with the other genotypes; i.e., those with 26 or more PARMs and those with NPARMs. There was a significant difference in male dominance between the patients with 25 PARM and the patients with the other genotypes ($P=0.045$).

Among the patients with 25 PARM, 12 of 19 patients (63%) developed hypoventilation during the neonatal period, and the other seven cases (37%) exhibited hypoventilation in the infantile period or childhood (Table 2). Four cases (the data for one case were not available) were diagnosed as CCHS in the neonatal period, and the median age of diagnosis was 4 months (IQR 1–33 months). No cases with 25 PARM had Hirschsprung disease.

All 73 cases carrying 26 or more PARMs or NPARMs exhibited apparent hypoventilation during the neonatal period. Ventilation support was required even during wakefulness in six cases carrying 26 PARM, 27 PARM and NPARMs except for the c.590delG mutation. Thirty-three of the 69 cases (the data for four cases were not available) were diagnosed as CCHS during the neonatal period, and the median age of diagnosis was one month (IQR 0–3 months). There was a significant difference in the age of diagnosis between the patients with 25 PARM and the other genotypes ($P<0.01$). There was a correlation between the length of the alanine repeats and the incidence of Hirschsprung disease ($r=0.327$, $P<0.01$) or the rate of complication of constipation without Hirschsprung disease ($r=0.370$, $P<0.001$). The patients with NPARMs except for the c.590delG mutation had Hirschsprung disease (Table 1).[27]

The median age of diagnosis of the patients with 25 PARM was 4 months, which was greater than that of the patients with other mutations. We were impressed that many patients carrying 25 PARM were mentally retarded and developmental quotients or intelligence quotients scores were assessed in the patients carrying 25 PARM as

Table 2 Clinical features of the cases with 25 polyalanine repeat expansion mutations

Case	sex	GA (wk)	Birth weight (g)	Apgar score 1 min/5 min	Age at presentation of CH	Age at diagnosis of CCHS	Ventilatory management (periods)	DQ or IQ (assessed method) age	Other clinical features
1	M	40	3046	9/na	1 mo	5 mo	CPAP (1 mo–10 mo) Tracheostomy and IMV (10 mo–4 yr) BiPAP (4 yr–)	DQ 99 (*K*-test) 5.6 yr	
2	M	39	2900	8/na	10 mo	15 yr	LTOT and IMV (10 mo–11 mo) Tracheostomy and IMV (11 mo–)	DQ 71 (Enjoji) 10 mo	
3	M	38	2902	9/10	<1 mo	1 mo	Intubation and IMV (<1 mo–)	na	
4	M	33	2282	8/9	<1 mo	<1 mo	BiPAP (<1 mo–)	IQ 85 (WISC-III) 8.1 yr	Familial case
5	F	38	3000	9/na	<1 mo	1.6 yr	Intubation & IMV (<1 mo–2 mo) Tracheostomy & IMV (2 mo–7 yr) BiPAP (7 yr–)	DQ 88 (WPPSI) 5.7 yr	
6	F	41	2786	9/10	<1 mo	1 mo	Intubation and IMV (day 7–1 mo) Tracheostomy and IMV (1 mo–)	DQ 117 (Enjoji) 3 yr	
7	M	37	na	na/na	1.2 yr	1.2 yr	HOT (1.2 yr–5 yr) BiPAP (5 yr–)	DQ 48 (*K*-test) 5 yr	Cor pulmonale reported case (ref. 25)
8	F	39	2758	9/10	<1 mo	2 mo	HOT (<1 mo–3 yr) BiPAP (3 yr–)	DQ 51 (*K*-test) 3.6 yr	
9	M	39	2802	na/na	1 mo	4 yr	BiPAP (1 mo–)	na	Pulmonary hypertension, cor pulmonale
10	M	39	2450	9/9	<1 mo	3 mo	CPAP (<1 mo–3 mo) Tracheostomy and IMV (3 mo–)	normally developed	Ventricular septal defect
11	M	40	3436	Asphyxia	<1 mo	<1 mo	HOT (<1 mo–3 yr) BiPAP (3 yr–)	IQ 60 (WISC-III) 8 yr	Pulmonary hypertension, constipation older brother of case 12
12	M	37	3312	5/6	<1 mo	3 mo	HOT (<1 mo–)	MR	Hypoxic-ischemic encephalopathy younger brother of case 11
13	M	36	2600	Asphyxia	<1 mo	10 yr	HOT (<1 mo–1 yr) BiPAP (10 yr–)	IQ 60 (WISC-III) 6.9 yr	Autism
14	M	na	na	na/na	<1 mo	11 yr	CPAP (<1 mo–1 mo) CPAP (11 yr–)	IQ 85 (WISC-III) 15.6 yr	
15	M	na	na	na/na	1 mo	na	BiPAP (6 mo–)	MR	Pervasive developmental disorder, familial case
16	M	37	2740	8/9	<1 mo	<1 mo	NPPV (<1 mo) HOT (4 yr–12 yr) BiPAP (12 yr–)	DQ 67 (*K*-test) 6 yr	Acute encephalopathy (12 yr 5 mo)
17	F	40	3050	9/10	3 yr	3 yr	BiPAP (3 yr–)	IQ <45 (WISC-III) 5.5 yr	
18	F	na	na	na/na	2 yr	2 yr	HOT (2 yr–2.8 yr) LTOT and BiPAP (2.8 yr–)	DQ 78 (*K*-test) 3.4 yr	
19	M	38	2705	9/10	<1 mo	<1 mo	Intubation and IMV (<1 mo–1 mo) Tracheostomy & IMV (1 mo–)	DQ 81 (Enjoji) 10 mo	Polyhydramnios

Abbreviations: BiPAP, biphasic positive airway pressure; CPAP, continuous positive airway pressure; CH, central hypoventilation; DQ, developmental quotient; Enjoji, Enjoji's analytical development test; GA, gestational age; HOT, home oxygen therapy; IMV, intermittent mandatory ventilation; IQ, intelligence quotient; *K*-test, the revised version of Kyoto Scale of psychological development; LTOT, long-term oxygen therapy; mo, month; MR, apparently mentally retarded but not scored by a standardized method; na, not available; NPPV, noninvasive positive pressure ventilation; WPPPSI, Wechsler Preschool and Primary Scale of Intelligence; WISC-III, Wechsler Intelligence Scale for Children-third edition; yr, year.

shown in Table 2, and 8 of the 19 cases (42%) with 25 PARM were complicated by mental retardation (Table 2).

DISCUSSION

We detected 92 cases of *PHOX2B* mutation-confirmed CCHS in Japan, estimated an incidence of >1 per 148 000 births. And in the cases carrying 25 PARM we found a male dominance and the frequent complication of mental retardation

There had not been previous epidemiological data about CCHS in Japan. Our 6-year analysis estimated the incidence of CCHS in Japan to be >1 case per 148 000 births, which was not significantly different from the estimated incidence of 1 case per 200 000 births that has been previously reported in France.[14] The cases that underwent genetic diagnosis seemed to represent nearly all of the patients in Japan because our facilities provide a unique genetic screening service for the molecular diagnosis of CCHS in Japan. However, this study was not based on clinical phenotype surveillance, and the cases that were only diagnosed clinically and those that were diagnosed molecularly in other countries were not included.

Notably, the number of male patients with 25 PARM was three times greater than the number of female patients with 25 PARM. The individuals carrying 25 PARM exhibited hypoventilation during the neonatal period or after the neonatal period and occasionally had no symptoms. In contrast, the patients with 26 or more PARMs did not show any sex differences and exhibited hypoventilation during the neonatal period. PARMs in *PHOX2B* have been shown to impair transcriptional function, and transcriptional impairment increases with expansion length for polyalanine repeat.[18] The 26 or more PARMs definitely impair the function of PHOX2B, have complete penetrance, and cause CCHS in both male and female infants. However, 25 PARM exhibited incomplete penetrance, which suggests that other genetic and/or epigenetic factors are possibly involved in the clinical onset of CCHS. One study reported that two women carrying 25 and 26 PARMs experienced improvements in hypoventilation

following the use of contraceptive pills that contained desogestrel, which is a potent progestin.[35] Progesterone derivatives are known to exert stimulatory effects on the respiration.[36] Prepubertal females have higher estrogen and lower testosterone and 17OH-progesterone levels than the prepubertal males.[37–39] In addition, in immature rats, there are gender differences in the estrogen levels in the cortex and hypothalamus despite the equivalent blood estrogen levels.[40] The possible mechanisms by which sex hormones might modulate breathing include direct or indirect effects on regulatory gene expression in respiratory neurons. Our study was small and thus cannot completely exclude sampling bias. The male dominance among patients with 25 PARM should be confirmed in more patients.

Eight cases with 25 PARM (42%) displayed apparent mental retardation. Before the disease-causing gene was identified, intellectual and cognitive deficits were commonly reported in CCHS patients.[41,42] Zeiko *et al.*[43] assessed the intellectual and cognitive abilities of 20 patients with PHOX2B mutation who had been diagnosed at neonates and placed under respiratory control. These authors found that the general Intelligence Index (full-scale intelligence quotients) of these patients was 84.9 ± 23.6 (mean $\pm$ s.d.) and that the PHOX2B genotype and disease severity indicators were unrelated to neurocognitive indices.[43] Our seven cases were observed to exhibit central hypoventilation in the infantile period or childhood. However, developmental or intellectual deficits were not always associated with the age of onset or the age at diagnosis of CCHS as shown in Table 2. Five of the eight patients with intelligence deficits received home oxygen therapy. Patients with 25 PARM might be under-diagnosed owing to subtle and or irregular hypoventilation and thus not receive appropriate ventilatory support, which might frequently cause the complication of mental retardation due to hypoxic encephalopathy. Early and definitive diagnoses based on *PHOX2B* analysis are convenient and useful for preventing neurological sequelae.

In addition, we observed depression at birth in 12 cases and polyhydramnios in 3 cases. The number of infants who exhibited low Apgar scores at 1 min was correlated with an increased number of polyalanine repeats, suggesting one of the symptoms of CCHS. The pregnancies of three cases (3.3%) were complicated by polyhydramnios. Polyhydramnios accompanied by a Chiari I malformation has been reported in one CCHS patient.[44] Faure *et al.*[4] observed esophageal dysmotility in seven cases via esophageal manometry and speculated that the underlying mechanism might be dysfunctions of the central structures that control swallowing. The complication of polyhydramnios might be derived from a dysfunction of swallowing control that is similar to that observed in congenital myotonic dystrophy.

In summary, we studied 92 Japanese patients with CCHS, estimated an incidence of CCHS and found a male dominance and the frequent complication of mental retardation in the cases carrying 25 PARM. Male sex is likely a predisposing factor for the patients carrying 25 PARM, who frequently had mental retardation likely because they presented subtle and or irregular hypoventilation and could not receive appropriate ventilation support following a definitive diagnosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Original Article

Breathing intolerance index in healthy infants

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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.

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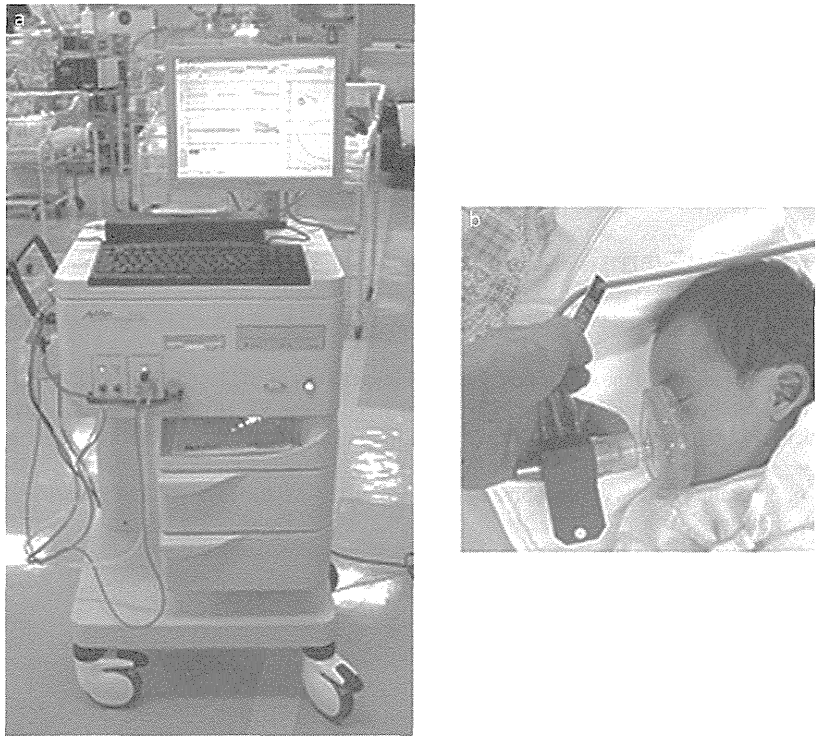

Fig. 1 (a) Aivision pulmonary function measuring system and (b) pneumotachometer with mask.

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

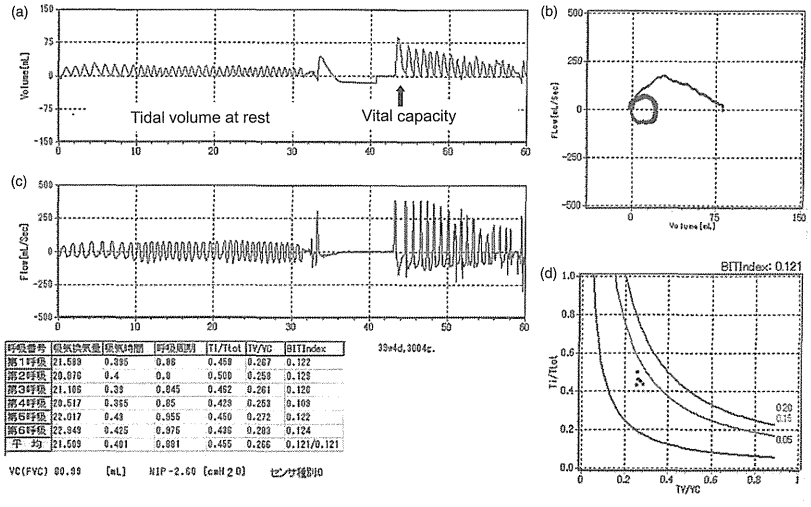

Fig. 2 (a–c) Volume, flows, and (d) breathing intolerance index (BITI). The six consecutive breath-by-breath data are plotted on the graph and averaged to obtain a BITI of 0.121.

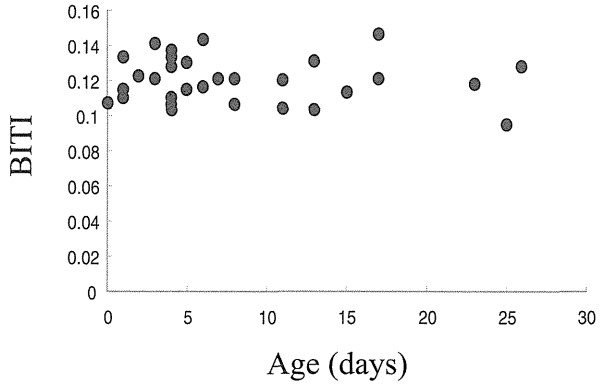

Fig. 3 Breathing intolerance index (BITI) vs age in healthy infants in the supine position.

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.

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Original Article

Decreased granulomatous reaction by polyurethane-coated stent in the trachea

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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.

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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:

Deformity (%) = (Stent external diameter without compression − Stent external diameter) × 100/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.

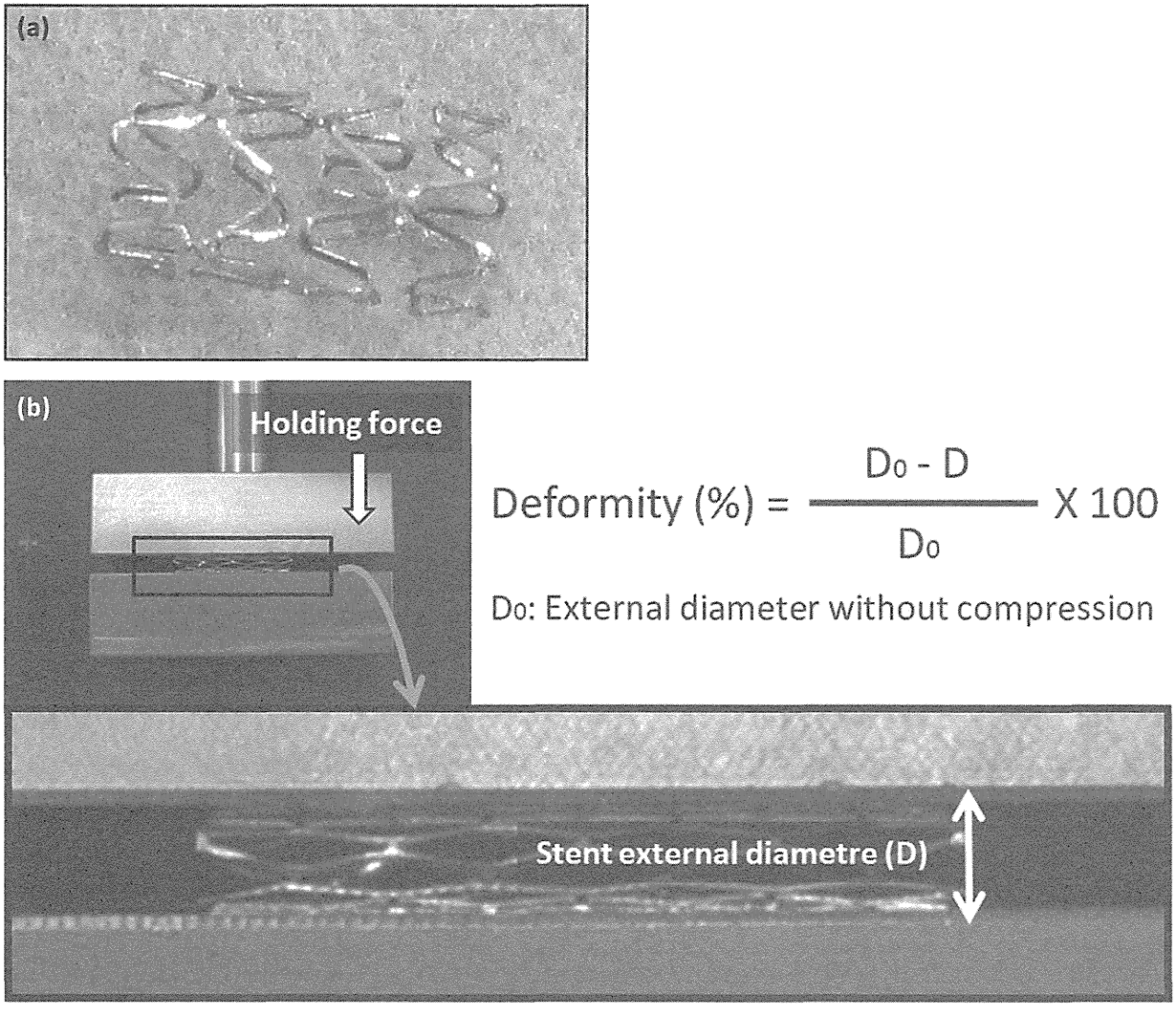

Fig. 1 New metallic stent and examination of holding force by compressor. (a) Picture of metallic stent. (b) Compressor and formula deformity. Deformity was defined as shortening as a fraction of external diameter of the stent. Holding force was measured in Newton units.

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

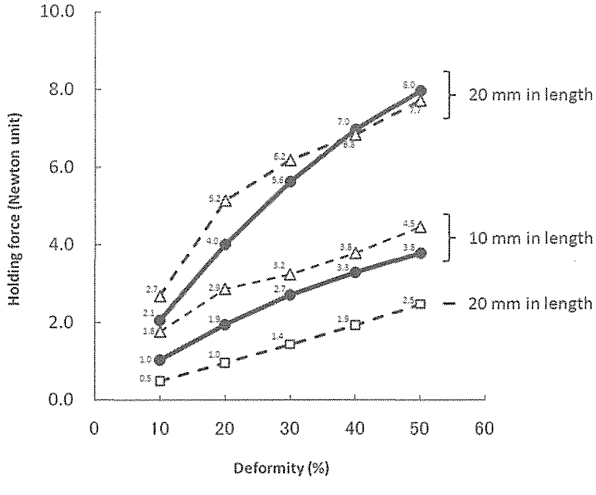

Fig. 2 Correlation between holding force and deformity. Data of holding force is labeled on the left-shoulder in each dot. The newly invented stent demonstrated similar mechanical properties to PLAMAZ metallic stents, while LUMINEX silicone stents showed significantly weaker holding force to other stents.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

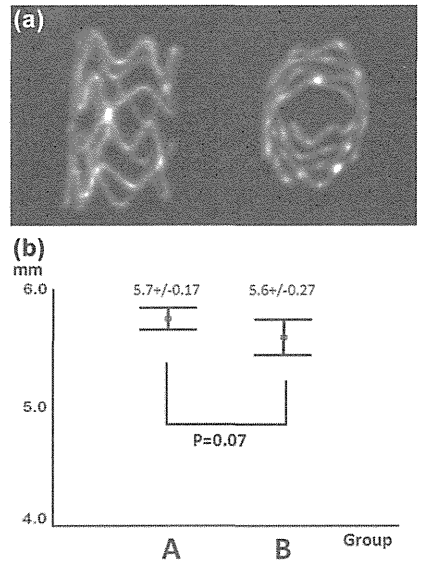

Fig. 3 Geometrical evaluation of implanted stents by multi-detector row computed tomography. (a) 3-D reconstructed image of implanted stents. No deformation was found and inner cavities were widely preserved in all stents. (b) Comparison of inner diameter of coated and non-coated stents (groups A and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

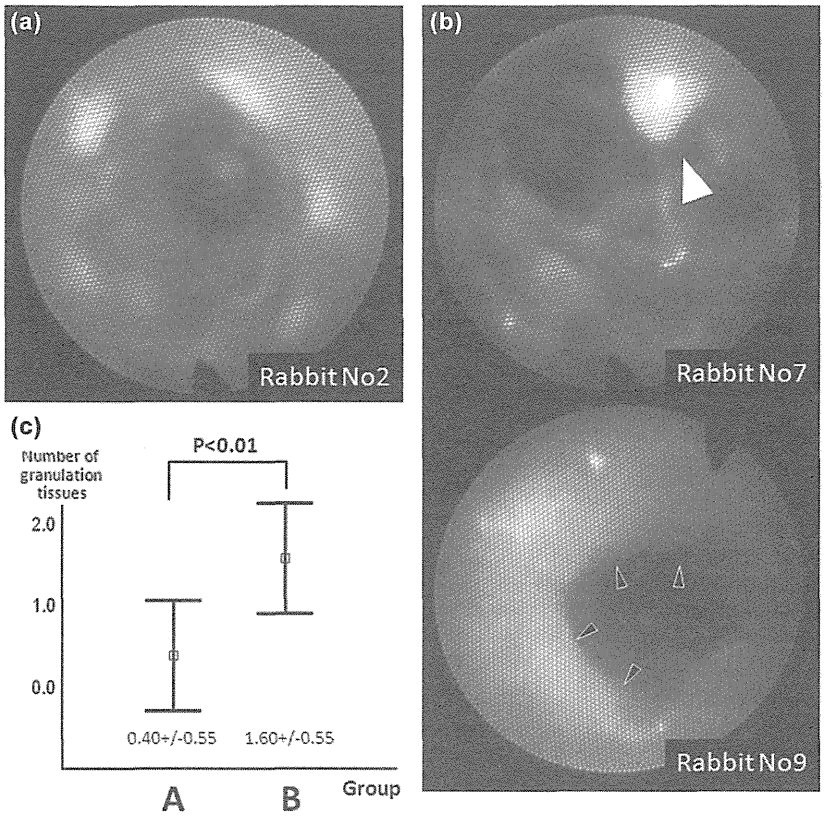

Fig. 4 Bronchofiberscopic images of coated and non-coated stents. (a) Image of implanted, coated stents. No obvious granulation tissues were found; metallic frame seems covered in tracheal membrane. (b) Image of implanted non-coated stents. Protrusion (white arrow) and significant bulging (black arrow) 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).

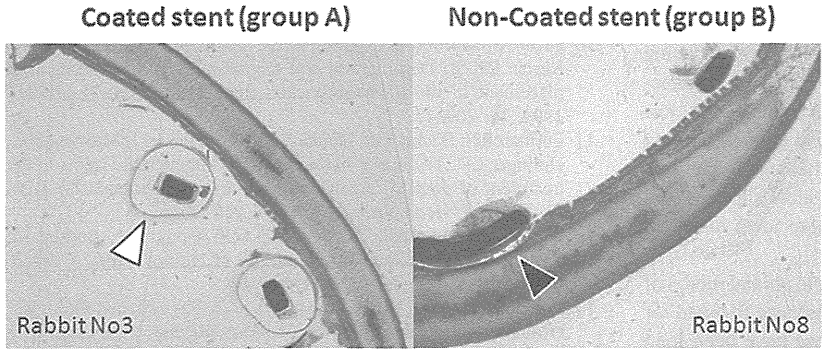

Fig. 5 Images of histological sections of implanted stents. (a) Coated stent, preserving tracheal structure (white arrow); (b) non-coated stent damage to tracheal epithelia and cartilage (black arrow).

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.

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