Functional evaluation of pallid mice with genetic emphysema

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Studies on pallid mice models of genetic emphysema have conventionally focused on morphological or biochemical evaluations. However, it is important to consider the functional aspects. We evaluated the exercise capacity and respiratory function in male pallid mice and male C57BL/6J mice at 3, 6, 12, and 15 months of age. The functional evaluations were conducted using a treadmill and a pulmonary function analysis device. The morphology of the lungs was analyzed on the basis of mean linear intercept (Lm) values. The body weights of the pallid mice at 12 and 15 months were significantly lower than those of the age-matched C57BL/6J mice. The pallid mice showed deterioration in exercise capacity from 6 months, as indicated by the trends in running distance. At 6, 12, and 15 months, the pallid mice showed significantly higher pulmonary compliance and significantly lower forced expiratory volume in 20 ms (FEV_{20ms})/vital capacity (VC) values in comparison with the corresponding values for the C57BL/6J mice. In the morphological analysis of the pallid mice, emphysema was detected from 12 months, and the mice showed a significantly larger Lm at 12 months. The exercise capacity and lung function in the pallid mice significantly deteriorated from 6 months, at which time no pathological changes in the lung were detected. The deterioration in the exercise capacity and pulmonary function preceded the microscopic morphological changes.

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No effective therapy for treating pulmonary emphysema has been established, and the number of patients with pulmonary emphysema and the costs associated with chronic obstructive pulmonary disease (COPD) continue to increase every year.^{1,2} To develop a treatment for pulmonary emphysema, many studies using animal models that involve intratracheal elastase instillation have been reported.^{3–5} Although such models are well established, they have some limitations. Because the injury is produced by a single overwhelming proteolytic insult delivered to the entire lung, the pathological and physiological features of these models are different from those of emphysema.⁶ The pallid mouse (C57 pa⁺/pa⁺) has a C57BL/6J origin, but it has a mutation in the pallid gene located on chromosome 2. This nonsense mutation at codon 69 of the gene hinders the normal secretion of α_1 -antitrypsin into circulation, consequently decreasing the inhibitory capacity of serum elastase.^{7–9} Pallid mice spontaneously develop emphysema, and are therefore a useful model for

emphysema studies. Morphological, biochemical, and genetic analyses have been performed on these mice.^{10–14} Morphological evaluations such as mean linear (Lm) intercept determinations are insufficient, and a functional evaluation is required. However, few studies have addressed the pulmonary function and exercise capacity in pallid mice.

In this study, we analyzed the exercise capacity and respiratory function in pallid and C57BL/6J mice by using a treadmill and a pulmonary function analyzer, respectively.

MATERIALS AND METHODS

Animals

The procedures used in this study were approved by the animal care committee of the University of Tokushima. Male C57BL/6J and male pallid mice (C57BL/6J, pa⁺/pa⁺) at 3, 6, 12, and 15 months of age were used. The C57BL/6J and pallid mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA) through Charles River Japan (Kanagawa, Japan).

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They were maintained in an animal house in a pathogen-free environment, with a 12-h light–dark cycle, and they were provided free access to water and food. The animals received humane care in compliance with the institutional and national guidelines (Japanese Ministry of Education, Culture, Sports, Science and Technology). The mice were weighed at regular intervals.

Treadmill Exercise

Each mouse was weighed immediately before each treadmill exercise capacity test. The distance (m), duration of shock (time of shock, TS; s), and number of shocks (NS) were measured by using the treadmill. The mice ran on a 32.0 cm × 4.8 cm double-lane treadmill (LE8709; Panlab sl, Cornella, Barcelona, Spain). Shock grids capable of delivering 0.2 mA of electric current were placed 5.5 cm from the rear of the cell to provide a stimulus that prompted the animals to run. The distance was defined as the mileage traveled by the mice without touching the shock grid. If the mice touched the grid for more than 1 s, the distance was not measured for that particular run. TS indicates the duration for which the mice touched the shock grid. NS is the number of times the mice touched the shock grid.

Before initiating the trials, the mice were placed on the treadmill and allowed to adapt to the surroundings. Familiarization runs were performed for approximately 2 min on a treadmill with an inclination of 10° and a speed that was gradually increased from 10 to 30 cm/s. Two trials were conducted for each mouse. The first trial was performed at a speed of 30 cm/s with a duration of 5 min and an inclination of 10°. After a 3-min interval, the second trial was performed under the same conditions. The best result of the two trials was accepted. The treadmill was set up on the basis of a pilot study.

Measurement of Lung Mechanics

To exclude the stress of repeated physical exertion, the respiratory function was measured at least 24 h after the treadmill study. Each mouse was weighed, anesthetized by an intraperitoneal injection of xylazine (8 mg/kg) and ketamine hydrochloride (80 mg/kg), tracheostomy was performed, and the trachea was cannulated with a 19-gauge Teflon needle. The cannula was connected to a computer-controlled, small-animal-ventilator system that is suitable for a battery of tests commonly used with cooperative humans (BioSystem for Maneuvers, SFT3410 with AUT6100 series; Buxco, Wilmington, NC, USA). The preclinical application of these tests involves a series of forced maneuvers on anesthetized subjects. The inflation of the lungs, occlusion, and slow and rapid evacuation of the lungs provide a range of diagnostic parameters, particularly in the study of COPD.

In detail, Buxco's Forced Maneuvers Systems provide comprehensive pulmonary function data for preclinical applications. For the measurement of functional residual capacity (FRC), subjects were required to be spontaneously

breathing under anesthesia. For the other measurements, subjects may be ventilated with a ventilator or they may be spontaneously breathing. Lung volumes and flow volume loops were measured by using a total-body plethysmograph equipped with software-operated solenoid valves connecting the tracheal catheter to outside air or to positive- and negative-pressure reservoirs. The intubated mice were placed in a dorsal position in the plethysmograph. These measurements were initiated at a point in time when the murine respiratory frequency was stable (180–220 per min).

FRC Measurement

Functional residual capacity was determined according to Boyle's law by using plethysmography. The dead space of the circuit used in this study was 0.66 ml. Breathing was controlled before each computerized measurement. An animal was used to control the volume history, and its breathing was synchronized with a computer. The mice were made to inspire 8 cmH₂O, and the expiration pressure was returned to the atmospheric pressure. This procedure was repeated 10 times. At the end of the last expiration of the volume history, a valve of a respiratory channel installed in the plethysmograph was shut. Following this, the valve was occluded for up to 8 s. The mice continued breathing voluntarily during this period. The occlusion of the valve was later removed. The quantity of change in the swelling of the lungs during spontaneous breathing after the respiratory tract occlusion (a flow wave pattern) was detected in the plethysmograph, and the internal pressure of the respiratory tract (a pressure wave pattern) at that instant was simultaneously measured. The quantity of air in the lungs at the end of expiration (FRC) is calculated on the basis of Boyle's law by using these values of pressure and volume, that is, $P_0V_0 = (P_0 + P_1)(V_0 + V_1)$, $V_0 = -V_1(P_0 + P_1)/P_1$, where P_0 is the pressure of the atmosphere; P_1 , the internal pressure change in the respiratory tract after respiratory tract occlusion; V_0 , FRC + 0.66 ml; and V_1 , quantity of volume change after respiratory tract occlusion. The mean of an FRC application was used for determining the respective FRC values.

Quasi-static Pressure Volume (PV) Measurement

Quasi-static PV determines the static properties of the lung and produces a volume–pressure curve of expiration. The total lung capacity (TLC), vital capacity (VC), residual lung volume (RV), and compliance were measured. The FRC test should be performed before these tests. At the end of the last expiration of volume history, the mice were forced to inspire up to an airway pressure of 30 cmH₂O (maximum mouth pressure) and hold it for a brief period (0.4 s). TLC was defined as the lung volume at a plateau airway-opening pressure of 30 cmH₂O after breath holding for 0.4 s. After 0.4 s of breath holding, the lungs were slowly expired to the RV at a pressure of –30 cmH₂O with an applied speed of 1 ml/s. The expiratory time must be at least 1 s. The quasi-static PV was

measured thrice, and the mean of each parameter was calculated.

Forced Expiratory Volume Measurement

The dynamic properties of the lungs are determined by measuring forced expiratory volume (FEV) values. This system delivers a flow–volume curve of expiration and computes the peak flow, flows at various volumes, and volumes at various times. At the end of the last expiration of volume history (FRC level), the mice inspired up to the maximum mouth pressure (30 cmH₂O, TLC level) and held it for a brief period (0.4 s). Following this, the mice expired to –50 cmH₂O as rapidly as possible. Quasi-static PV and FEV were measured thrice, and the mean of each parameter was calculated.

Lung Histology and Morphological Quantitation of Emphysema

After measuring the respiratory function, a laparotomy and thoracotomy were performed under anesthesia. The left atrium was dissected, and the lungs were perfused with saline through the pulmonary artery, following which the lungs were removed en bloc with the heart and fixed with 10% phosphate-buffered formalin solution supplied through the trachea at a constant pressure of 15 cmH₂O.

The left lungs were used for morphological analysis. Because the left lung has only one lobe, it is easy to determine the cutting positions. The left lungs were horizontally cut into four equal parts. An imaginary longitudinal line was drawn from the apex to the base of the lung, and it was divided into four equal parts. The specimens obtained were embedded in paraffin. Each caudal surface of the first, second, and third parts from the apex of the lung, which were defined as the upper, middle, and lower fields, respectively, was cut into 7- μ m-thick sections and stained with hematoxylin and eosin.

The Lm method was used to quantify the alveolar spaces.¹⁵ Briefly, 5 fields under a light microscope at $\times 200$ magnification were randomly selected from each specimen (ie, 15 fields in each left lung sample), and Lm was calculated on the basis of the total number of alveolar intercepts encountered along the length of a right-angled crosshair (500 μ m).

Statistical Analysis

All values are expressed as mean $\pm$ s.d. Statistical analyses were performed by using Student's *t*-test. For multiple comparisons, a one-way analysis of variance (ANOVA) was used along with the Bonferroni correction (SPSS software, version 11.0.1). $P < 0.05$ was considered to be statistically significant.

RESULTS

Body Weight

In comparison with the C57BL/6J mice, the pallid mice had significantly lower body weights at 12 and 15 months of age

Figure 1 Body weight changes. The body weights of the pallid mice did not increase from 12 months of age. The body weights of the pallid mice at 12 and 15 months of age were significantly lower than the corresponding weights of the C57BL/6J mice. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$. The body weights of both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The body weights of the C57BL/6J mice at 3 months of age were significantly lower than those of the C57BL/6J mice at 6 months ($P < 0.05$), 12 months ($P < 0.01$), and 15 months ($P < 0.01$) of age. The body weights of the C57BL/6J mice at 6 months of age were significantly lower than those of the C57BL/6J mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. The body weights of the pallid mice at 3 months of age were significantly lower than those of the pallid mice at 6 months ($P < 0.01$), 12 months ($P < 0.01$), and 15 months ($P < 0.01$) of age.

(Figure 1). The body weights of the C57BL/6J mice increased until 12 months of age. The nutritional status of the C57BL/6J mice seemed to be adequate at all ages, whereas the nutritional status of the pallid mice seemed to deteriorate after 12 months of age.

Treadmill Running Test Results: Exercise Capacity

Parameters of distance

The pallid mice covered a significantly smaller distance on the treadmill than did the C57BL/6J mice at 6, 12, and 15 months of age. The pallid mice showed a progressive deterioration in the exercise capacity from 6 months of age, indicated by the distance covered by the mice. In contrast, the C57BL/6J mice maintained their exercise capacity to an advanced age (Figure 2a). Individual differences among the pallid mice increased at 15 months of age.

Time of shock and number of shock

Mice with poor exercise capacities ran in the vicinity of the shock grid; accordingly, TS and NS increased simultaneously. At 6, 12, and 15 months of age, the pallid mice demonstrated significantly higher values of TS and NS than the C57BL/6J mice (Figure 2b and c). The TS and NS values of the pallid

Figure 2 (a) Distance on treadmill analysis. The pallid mice covered a significantly lesser distance on the treadmill in comparison with distances covered by the C57BL/6J mice at 6, 12, and 15 months of age. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$; **pallid vs C57BL/6J, $P < 0.01$. The distances for both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The distances covered by the C57BL/6J mice at 3 months of age were significantly higher than those covered by the C57BL/6J mice at 6 months ($P < 0.01$) and 12 months ($P < 0.01$) of age. The distances covered by the pallid mice at 15 months of age were significantly lower than those covered by the pallid mice at 3 months ($P < 0.01$), 6 months ($P < 0.01$), and 12 months ($P < 0.05$) of age. (b) Time of shock (TS) on treadmill analysis. (c) Number of shock (NS) on treadmill analysis. The pallid mice demonstrated significantly higher TS and NS values at 6, 12, and 15 months of age in comparison with the corresponding values of C57BL/6J mice. The values are represented as the mean $\pm$ s.d. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$; **pallid vs C57BL/6J, $P < 0.01$. TS and NS of both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The TS and NS for the C57BL/6J mice at 3 months of age were significantly lower than those of the C57BL/6J mice at 6 months ($P < 0.05$) and 12 months ($P < 0.05$) of age. TS of the pallid mice at 3 months of age was significantly lower than that of the pallid mice at 15 months ($P < 0.05$) of age. NS values of the pallid mice at 15 months of age were significantly higher than those of the pallid mice at 3 months ($P < 0.01$) and 6 months ($P < 0.05$) of age.

mice worsened with age. These results indicate a decrease in movement. The pallid mice showed low exercise capacity from 6 months of age, which corresponded to a decrease in the distance covered on the treadmill. Individual differences in TS and NS of the pallid mice increased after 6 months of age.

Pulmonary Function Test

RV, FRC, TLC, VC, and compliance

We did not observe any significant differences in FRC and RV values of the pallid and C57BL/6J mice at any age. Among the aged mice, the mean value of TLC in the pallid mice was

higher than that in the C57BL/6J mice; however, there was no significant difference between the values for the C57BL/6J and pallid mice (Table 1).

In comparison with the C57BL/6J mice, the pallid mice showed significantly higher VC and compliance at 6, 12, and 15 months of age (Figures 3 and 4). Significant differences in the VC and compliance between the two groups appeared from 6 months of age. The VC of the C57BL/6J mice and the pallid mice showed a significant increase between 3–6 months and 12–15 months of age. The compliance showed a significant increase between 3–6 months and 12 months of age in the C57BL/6J mice, and between 3–6 months and 12–15 months of age in the pallid mice (Figures 3 and 4).

Table 1 FRC, RV, and TLC

Parameters	Strain	Age (months)			
		3	6	12	15
RV (ml)	C57BL/6J	0.22 ± 0.06	0.24 ± 0.05	0.27 ± 0.05	0.22 ± 0.05
	Pallid	0.19 ± 0.06	0.22 ± 0.05	0.22 ± 0.07	0.23 ± 0.08
FRC (ml)	C57BL/6J	0.31 ± 0.05	0.33 ± 0.05	0.37 ± 0.05	0.36 ± 0.05
	Pallid	0.31 ± 0.06	0.36 ± 0.08	0.38 ± 0.04	0.39 ± 0.08
TLC (ml)	C57BL/6J	0.89 ± 0.13	0.96 ± 0.19	1.18 ± 0.13	1.18 ± 0.13
	Pallid	0.91 ± 0.16	1.09 ± 0.14	1.28 ± 0.14	1.31 ± 0.17

FRC, functional residual capacity; RV, residual volume; TLC, total lung capacity. The values of FRC, RV, and TLC did not differ significantly between the C57BL/6J and the pallid mice at any age.

The FRC, RV, and TLC values of both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The TLC of the C57BL/6J mice at 6 months of age had a significantly lower value than those of the C57BL/6J mice at 12 months ($P < 0.05$) and 15 months ($P < 0.05$) of age. The TLC of the C57BL/6J mice at 3 months of age had a significantly lower value than those of the C57BL/6J mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. The TLC of the pallid mice at 6 months of age had a significantly lower value than that of the pallid mice at 15 months ($P < 0.05$) of age.

The TLC of the pallid mice at 3 months of age had a significantly lower value than those of the pallid mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. Values are represented as the means ± s.d.

Forced vital capacity

The forced vital capacity (FVC) values in the pallid mice tended to be higher than those in the C57BL/6J mice at all ages. In both strains, the growth-associated pattern of change in the FVC values showed the same trend as that in the VC values. At 12 months of age, the pallid mice showed significantly higher FVC values than the C57BL/6J mice (Figure 5a). The FVC showed a significant increase between 3–6 months and 12–15 months of age in the C57BL/6J mice and the pallid mice.

FEV_{20ms}/FVC

FEV_{20ms} corresponds to the FEV in 1 s (FEV₁) in humans; therefore, FEV_{20ms}/FVC (corresponding to the Gaensler index in humans) and FEV_{20ms}/VC (corresponding to the Tiffeneau index in humans) were used as an index of severity of airflow limitation. In the C57BL/6J and pallid mice, the FEV_{20ms}/FVC decreased with age. Moreover, the FEV_{20ms}/FVC in the pallid mice at 3 months of age was demonstrated to be significantly higher than that at all other ages. ANOVA did not reveal any statistically significant differences in the C57BL/6J mice (Figure 5b).

FEV_{20ms}/VC

At 6, 12, and 15 months of age, the FEV_{20ms}/VC in the pallid mice was significantly lower than that in the C57BL/6J mice. In the pallid mice, the FEV_{20ms}/VC at 3 months was significantly higher than that at 12 and 15 months. ANOVA did

Figure 3 Vital capacity (VC). The pallid mice showed significantly higher VC in comparison with the corresponding values for the C57BL/6J mice at 6, 12, and 15 months of age. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$; **pallid vs C57BL/6J, $P < 0.01$. The VC values of both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The C57BL/6J mice at 3 and 6 months of age showed significantly lower VC in comparison to the corresponding values for C57BL/6J mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. The VC values of the pallid mice at 3 months of age were significantly lower than those of the pallid mice at 6 months ($P < 0.05$), 12 months ($P < 0.01$), and 15 months ($P < 0.01$) of age. The VC values of the pallid mice at 6 months of age were significantly lower than those of the pallid mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age.

not reveal any statistically significant differences in the C57BL/6J mice (Figure 5c).

Lm

Representative histological sections from the left lungs of C57BL/6J and pallid mice have been presented (hematoxylin and eosin staining, $\times 200$) (Figure 6A). The pallid mice began to show air space enlargement from 12 months of age (e, f).

The pallid mice showed a significantly higher Lm than the C57BL/6J mice at 12 months of age. The Lm of the C57BL/6J mice at 15 months of age was significantly higher than that of the C57BL/6J mice at 3 and 6 months of age. The Lm of the pallid mice at 12 and 15 months of age was significantly higher than that of the pallid mice at 3 and 6 months of age (Figure 6B).

At all ages, we did not observe any bias in the Lm within each field (upper, middle, and lower) in any of the age-matched animals. ANOVA did not reveal any statistically significant differences in the C57BL/6J and the pallid mice (Figure 6C).

DISCUSSION

The pallid mice that developed emphysema spontaneously showed a loss in pulmonary function and exercise capacity from an early age, when no morphological changes were observed under a light microscope.

Figure 4 Pulmonary compliance. The pulmonary compliance of the pallid mice at 6, 12, and 15 months of age was significantly higher than the corresponding values of the C57BL/6J mice. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$; **pallid vs C57BL/6J, $P < 0.01$. The compliance values of both strains were analyzed by a one-way analysis of variance along with Bonferroni correction. The C57BL/6J mice at 3 months of age showed a significantly lower compliance in comparison with the corresponding values for C57BL/6J mice at 12 months ($P < 0.05$) and 15 months ($P < 0.05$) of age, and the C57BL/6J mice at 6 months of age showed a significantly lower compliance in comparison with the corresponding values of C57BL/6J mice at 12 months ($P < 0.05$) of age. The pallid mice at 3 and 6 months of age showed a significantly lower compliance in comparison with the corresponding values of pallid mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age.

Martorana *et al*^{16,17} reported age-related body weight changes in pallid and Tsk mice. In the current study, the body weights of the pallid mice were significantly lower than those of the C57BL/6J mice from 12 to 15 months of age. After 6 months of age, the gain in weight of the pallid mice reduced. The period during which there was a significant difference in the body weight coincided with the period of morphological changes. This suggests that the body weight of pallid mice is an indirect parameter of the progression of emphysema. As observed in COPD patients, an increase in the resting energy expenditure for a markedly elevated rate of breathing may cause the deterioration of weight gain in pallid mice.^{18–20} The consequence of this deterioration may be reduced muscle strength, including weakness of both the inspiratory and expiratory muscles.²¹

Experimental exercise models in mice use either voluntary or forced activity. Forced models, such as treadmill running, use aversive stimuli to induce exercising.²² This system has the advantage that the animals can be made to exercise at reproducible distances and speeds.²³ Many groups have used forced treadmill running to observe phenotypic differences between mice.^{24–26} Therefore, treadmill running is a useful method for evaluating cardiopulmonary function as well. The gene mutations of pallid mice are characterized by immature platelet dense granules, defective secretion of kidney lysosomal enzymes, abnormal otolith formation, and

a pale coat color caused by fewer and less mature melanosomes;^{27–30} however, disorders of the musculoskeletal system, nervous system, and cardiac function have not been reported. The exercise capacity of pallid mice can be evaluated with treadmill running, because pallid mice do not exhibit exercise resistance.

Before the appearance of morphological changes, the pulmonary compliance of the pallid mice significantly decreased at 6 months of age in comparison with the corresponding values for the control mice. In the control mice, pulmonary compliance significantly increased between 3–6 and 12 months of age; therefore, this change is thought to be a physiological change that occurs with growth. In the pallid mice, pulmonary compliance significantly increased between 3–6 and 12–15 months of age, and was significantly higher than that of the C57BL/6J mice at 6, 12, and 15 months of age. Therefore, it is postulated that the increase in pulmonary compliance in the pallid mice was caused by physiological and pathological factors. The concentration of elastin in the lungs of the pallid mice after 12 months of age is significantly lower than that in the age-matched controls.¹⁰ Elastase is observed on elastin within the alveolar walls of pallid mice from 2 months, and it increases with age, reaching high values at 12 and 16 months of age, which corresponds to the appearance of parenchymal destruction.¹² These data suggest that a progressive increase in elastase and decrease in elastin cause emphysematous lesions in pallid mice. It is possible that the increase in compliance observed in the pallid mice depends on the decrease in lung elastin, which has already been reported.

In addition to the pulmonary function test, the exercise capacity of the pallid mice showed a clear deterioration from 6 months of age. This change occurred before the manifestation of morphological changes. However, further examinations are needed to clarify the mechanism of this exercise limitation in pallid mice.

In this study, the $FEV_{20\text{ms}}/VC$ in the pallid mice was significantly lower than that in the control mice at 6, 12, and 15 months of age; however, the differences in $FEV_{20\text{ms}}/FVC$ were not significant. There are several explanations for these results. In a clinical setting, the presence of airflow limitation is recognized by a reduction in the ratio of $FEV_{1.0}$ to VC or FVC, and this $FEV_{1.0}/VC$ ratio is a relatively sensitive index of mild COPD. In moderate to severe disease, the severity of airflow limitation is best assessed by the FEV_1 in relation to the reference values.³¹ The $FEV_{1.0}/VC$ ratio, that is, the Tiffeneau index, is considered to be a more sensitive index of airflow limitation. The FVC is underestimated if the time allowed for lung emptying at low lung volumes is insufficient, in the cases where the emptying rate is determined by airflow limitation.³² Mouse lungs have a very small capacity, and in the current study, the time for emptying the lungs was 200 ms, which is very short. The FEV was passively measured by absorbing air in the airway of mice with a negative pressure of $-50\text{ cmH}_2\text{O}$ for 1 s. In comparison with

Figure 5 (a) Forced vital capacity (FVC). The pallid mice tended to have a higher FVC than the C57BL/6J mice at all ages. The FVC showed the same trend as the vital capacity and the pallid mice showed a significantly higher FVC than the C57BL/6J mice only at 12 months of age. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$. The FVC values of both strains were analyzed by one-way analysis of variance along with Bonferroni correction. The C57BL/6J mice at 3 and 6 months of age showed a significantly lower FVC in comparison with the corresponding values for C57BL/6J mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. The pallid mice at 3 months of age showed a significantly lower FVC in comparison with the corresponding values for the pallid mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. The pallid mice at 6 months of age showed a significantly lower FVC in comparison with the corresponding values for pallid mice at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. (b) Forced expiratory volume (FEV)_{20ms}/FVC. The values of the FEV_{20ms}/FVC did not differ significantly between the C57BL/6J and the pallid mice at any age. The number of mice is shown in parentheses. The FEV_{20ms}/FVC values of both strains were analyzed by one-way analysis of variance along with Bonferroni correction. The FEV_{20ms}/FVC deteriorated with age in the pallid mice, and the pallid mice at 3 months of age showed significantly higher FEV_{20ms}/FVC in comparison with the corresponding values for pallid mice at 6 months ($P < 0.05$), 12 months ($P < 0.01$), and 15 months ($P < 0.01$) of age. The one-way analysis of variance did not reveal any statistically significant differences in the C57BL/6J mice. (c) FEV_{20ms}/VC. The pallid mice demonstrated a significantly lower FEV_{20ms}/VC (Tiffeneau index) than the C57BL/6J mice at 6, 12, and 15 months of age. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$; **pallid vs C57BL/6J, $P < 0.01$. The FEV_{20ms}/VC values of both strains were analyzed by one-way analysis of variance along with Bonferroni correction. In the pallid mice, the FEV_{20ms}/VC at 3 months of age was significantly higher than those at 12 months ($P < 0.01$) and 15 months ($P < 0.01$) of age. One-way analysis of variance did not reveal any statistically significant differences in C57BL/6J mice.

the VC values, the FVC values in the pallid mice were less by approximately 0.03–0.05 ml in all the age groups. The values of the air-trapping index (ATI, (VC–FVC)/VC) in the pallid mice tended to be higher than those in the C57BL/6J mice of all ages (data not shown). The peripheral air ducts of pallid mice with emphysematous lesions may become frail and cause air trapping.

The current results demonstrated that Lm was normal at 3 and 6 months, when the mice began to develop functional changes. After 12 months of age, light microscopy revealed emphysematous changes in the pallid mice; however, the Lm

of pallid mice was significantly wider than that of the control mice only at 12 months of age. Keil *et al*¹³ measured Lm at 1, 12, and 24 months of age in pallid and C57BL/6 mice and reported a significant difference at 12 months but not at 24 months. The current results are consistent with their data. Martorana *et al*¹⁰ reported that significant differences in Lm between pallid and C57BL/6J mice were not observed at 12 and 28 months of age, and that it was detected at 8 months of age with contrary results. Lm has been used as an index to indicate expanding alveolar spaces, and it is often used in studies that use pallid mice as an emphysema model.^{10–14} It is

Figure 6 Histological section (A), Lm (B), and Lm in the upper, middle, and lower fields in the left lung (C). (A) The representative histological sections from the left lung of C57BL/6J (a–c) and pallid mice (d–f) are presented (hematoxylin and eosin staining, $\times 200$). The pallid mice began to show air space enlargement from 12 months of age (e and f). (B) The pallid mice at 12 months of age showed a significantly higher Lm than the age-matched C57BL/6J mice. The number of mice is shown in parentheses. *Pallid vs C57BL/6J, $P < 0.05$. The Lm values of both strains were analyzed by one-way analysis of variance along with Bonferroni correction. The Lm values of C57BL/6J mice at 15 months of age were significantly higher than those of the C57BL/6J mice at 3 months ($P < 0.05$) and 6 months ($P < 0.05$) of age. The Lm values of the pallid mice at 12 months of age were significantly higher than those of the pallid mice at 3 months ($P < 0.01$) and 6 months ($P < 0.01$) of age. The Lm values of the pallid mice at 15 months of age were significantly higher than those of the pallid mice at 3 months ($P < 0.01$) and 6 months ($P < 0.01$) of age. At all ages, neither strain showed a bias of the Lm within each field (upper, middle, and lower) at the same age. (C) One-way analysis of variance did not reveal any statistically significant differences in the C57BL/6J and the pallid mice.

thought that Lm does not always reflect the morphological changes that are observed in the patchy emphysematous lungs of pallid mice. Regarding this type of emphysematous lesion in the targeted area of Lm, it is postulated that normal alveolar areas reduce the distribution of emphysematous lesions, resulting in an increase in the value of Lm.

The α_1 -antitrypsin-deficient pallid mice, which suffer from emphysema in the period from maturation to advanced age, are suitable as emphysema models in emphysema-related

studies. This study showed that the exercise capacity and lung function of pallid mice significantly deteriorated from 6 months of age, with no detection of pathological changes in light microscopic analysis of the lung.

In addition to the knowledge regarding the biochemical, morphological, and functional aspects of pallid mice as an emphysema model, which have been previously reported,^{10–14} new findings were revealed in this functional study on mice. Therefore, this model for the evaluation of

lung function tests is considered to positively contribute to future research on emphysema.

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