Respiratory Physiology & Neurobiology 248 (2018) 48–54

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Loss of CDKL5 disrupts respiratory function in mice

Kun-Ze Lee[a,b,c,d,e,*], Wenlin Liao[f,g]

[a] Department of Biological Sciences, National Sun Yat-sen University, Kaohsiung 804, Taiwan
[b] Center for Neuroscience, National Sun Yat-sen University, Kaohsiung 804, Taiwan
[c] Institute of Medical Science and Technology, National Sun Yat-sen University, Kaohsiung 804, Taiwan
[d] Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung 804, Taiwan
[e] Doctoral Degree Program in Marine Biotechnology, National Sun Yat-sen University and Academia Sinica, Taiwan
[f] Institute of Neuroscience, National Cheng-Chi University, Taipei 116, Taiwan
[g] Research Center for Mind, Brain and Learning, National Cheng-Chi University, Taipei 116, Taiwan

ARTICLE INFO

Keywords:
CDKL5
Respiration
Tidal volume
Hypoxia
Hypercapnia

ABSTRACT

Cyclin-dependent kinase-like 5 (*CDKL5*) is an X-linked gene encoding a serine-threonine kinase that is highly expressed in the central nervous system. Mutations in *CDKL5* cause neurological and psychiatric symptoms, including early-onset seizures, motor dysfunction, autistic features and sleep breathing abnormalities in patients. It remains to be addressed whether loss of CDKL5 causes respiratory dysfunction in mice. Here, we examined the respiratory pattern of male *Cdkl5*$^{-/y}$ mice at 1–3 months of age during resting breathing and respiratory challenge (i.e., hypoxia and hypercapnia) via whole body plethysmography. The results demonstrated that the resting respiratory frequency and tidal volume of *Cdkl5*$^{-/y}$ mice was unaltered compared to that of WT mice at 1 month of age. However, these mutant mice exhibit transient reduction in tidal volume during respiratory challenge even the reduction was restored at 2 months of age. Notably, the sigh-breathing pattern was changed in *Cdkl5*$^{-/y}$ mice, showing a transient reduction in sigh volume at 1–2 month of age and long-term attenuation of peak expiratory airflow from 1 to 3 month of age. Therefore, loss of CDKL5 causes breathing deficiency, supporting a CDKL5-mediated regulation of respiratory function in mice.

1. Introduction

Cyclin-dependent kinase-like 5 (CDKL5) is a serine/threonine kinase encoded by *CDKL5* gene on Xp22 (Montini et al., 1998). Down-regulation of CDKL5 protein, which is localized at both pre- and post-synaptic structures, significantly reduces the number of excitatory synapses and miniature excitatory postsynaptic currents (Ricciardi et al., 2012). In addition, dendritic arborization of *in vitro* cultured neurons and *in vivo* neonatal rat cortex was impaired when CDKL5 expression was abolished (Chen et al., 2010). These results suggest that CDKL5 plays a critical role in neuronal morphogenesis and synaptic stability. Clinically, *CDKL5* mutations have been associated with various symptoms of neurological dysfunction, including early-onset seizure, mental retardation and stereotypic movements (Archer et al., 2006; Kobayashi et al., 2016), as well as breathing and sleep abnormalities (Hagebeuk et al., 2013). A recent study in mouse model demonstrated that the occurrence of sleep apnea was more frequent in adult *Cdkl5* knockout mice compared to wild type (WT) controls, but their resting breathing pattern was quite normal (Lo Martire et al., 2017). Given that CDKL5 is highly expressed in the brain during the early postnatal stage and gradually down-regulated with age (Rusconi et al., 2008), we thus hypothesize that the breathing pattern of *Cdkl5* knockout mice may start to decline prior to the adulthood. Here we first examined the breathing patterns of *Cdkl5* null mice at 1, 2 and 3 months of age, and then evaluated the respiratory function of *Cdkl5* null mice in response to respiratory challenge (e.g., sigh, hypoxia and hypercapnia) which has been demonstrated to reveal or exaggerate respiratory deficiency in some models of neurological disorders (Lee et al., 2011; Nichols et al., 2015; Voituron et al., 2010).

2. Material and methods

2.1. Animals

Male *Cdkl5* null mice (*Cdkl5*$^{-/y}$) were generated by crossing wild-type C57BL/6J male mice (National Laboratory Animal Center, Taiwan) with heterozygous *Cdkl5* females [*Cdkl5*$^{+/-}$, B6.129(FVB)-*Cdkl5*$^{tm1.1Joez}$/J, The Jackson Laboratory] in which the kinase domain of CDKL5 is truncated by insertion of a premature STOP codon at exon 6 (Wang et al., 2012). A total of 9 *Cdkl5*$^{-/y}$ and 10 littermate WT controls

* Corresponding author at: Department of Biological Sciences, College of Science, National Sun Yat-sen University, Kaohsiung 804, Taiwan.
E-mail address: kzlee@mail.nsysu.edu.tw (K.-Z. Lee).

https://doi.org/10.1016/j.resp.2017.11.010
Received 14 October 2017; Received in revised form 27 November 2017; Accepted 29 November 2017
Available online 05 December 2017
1569-9048/

were used. All mice were bred in the individually ventilated cage system (Alternative Design, USA) and housed at 22 ± 2 °C and 60 ± 10% humidity under a 12-h light-dark cycle (light on 08:00–20:00) with freely accessing to food and water. All experimental procedures were performed in accordance with the guidelines for experimental animals and approved by the Institutional Animal Care and Use Committee at the National Sun Yat-sen University.

2.2. Genotyping

The mice were weaned and ear-tagged at postnatal days 21–23, and then genotyped as previously described (Kao et al., 2015). Briefly, one microliter of genomic DNA extracted from the tail tissues was used for each PCR reaction containing primers of FW (5′-CCACCCTCTCAGTAA GGCAG-3′) and RV (5′-GTCCTTTTGCCACTC AATTC-3′). PCR amplification was carried out at 94 °C for 5 min followed by 35 cycles at 94 °C for 30 s, 64 °C for 40 s and 72 °C for 60 s. The PCR products of 653 bp and 305 bp correspond to the WT and null allele, respectively.

2.3. Whole body plethysmography

The breathing pattern (e.g., respiratory frequency, tidal volume, minute ventilation) of unanesthetized mice (WT, n = 10; *Cdkl5*$^{-/y}$, n = 9) was measured by the whole body plethysmography system (Buxco® Whole Body Plethysmography, Data Sciences International) at 1 month (34 ± 1 days), 2 months (61 ± 1 days) and 3 months (89 ± 1 days) of age. On the day of the experiment, the airflow, temperature and humidity of the animal chamber (#PLY4211, 450 ml) were calibrated using the standard procedure indicated by the manual, and the gain of the amplifier was set at 8x. The rectal temperature of each mouse was measured and the value was imported into the system to calibrate the respiratory parameters. The animal was then placed in the chamber and exposed to normoxic gas (21% O_2, 79% N_2) for 60–90 min by flushing with compressed gas (1 l/min). To evaluate whether the breathing pattern was altered during respiratory challenge, hypoxic gas (10% O_2, 4% CO_2, 86% N_2; 1 l/min) was introduced into the chamber for 10 min followed by 10 min of compressed air. An additional 10 min of hypercapnic gas (21% O_2, 7% CO_2, 72% N_2; 1 l/min) was provided after recovery from the hypoxic treatment. These two types of respiratory challenge have been used to increase the respiratory drives in unaneshtetized animals in our previous reports (Lee et al., 2017; Lee et al., 2014).

Table 1
The body weight (g) of the mice used in this study.

	1 month	2 month	3 month
WT (n = 10)	19.7 ± 0.6	25.9 ± 0.3**	28.6 ± 0.5***
Cdkl5$^{-/y}$ (n = 9)	18.5 ± 0.5	23.5 ± 0.4*	25.8 ± 0.5**,****

* P < 0.05 vs. 1 month.
** P < 0.01 vs. 1 month.
*** P < 0.01 vs. 1 and 2 month.
**** P < 0.05 significant difference between WT mice and *Cdkl5*$^{-/y}$ mice.

2.4. Data analysis

The respiratory parameters (e.g., respiratory frequency, tidal volume and minute ventilation) were calculated using FinePointe software (Data Sciences International) and exported to an Excel file in a 10 s bin. The data collected during stable normoxic breathing were averaged over 10 min to obtain a baseline value. Because the tidal volume and minute ventilation are positively correlated with the body weight, both data were presented as an absolute value and the value normalized with the body weight to examine whether different body weight between WT and *Cdkl5*$^{-/y}$ may influence the tidal volume and minute ventilation. The hypoxic and hypercapnic responses are represented by the data averaged during the last 2 min of hypoxia or hypercapnia, respectively. These data were presented as an absolute value and a percentage of the baseline value (% BL).

To compare "sigh" breathing pattern (Voituron et al., 2010) between WT and *Cdkl5*$^{-/y}$ mice, the box airflow tracing in a subset of animals (WT, n = 8; *Cdkl5*$^{-/y}$, n = 7) was exported to an analog-to-digital converter (Power1401, Cambridge Electronic Design Limited) at sampling frequency of 1000 Hz. The occurrence and pattern (i.e., inspiratory duration, expiratory duration, sigh volume and peak respiratory airflow) of sigh was detected and analyzed by a Spike 2 software during the 10 min stable normoxic breathing period. Because the box airflow did not reflect the absolute value of respiratory airflow generated from the mice, the sigh volume and peak respiratory airflow was presented as an arbitrary unit (a.u.).

A two-way mixed design analysis of variance (ANOVA) followed by a Student-Newman-Keuls post-hoc test was used to analyze the body weight and respiratory parameters [factor one: animal group (WT vs. *Cdkl5*$^{-/y}$); factor two: age (1, 2 and 3 months) or breathing condition (baseline, hypoxia and hypercapnia)]. All data are shown as the mean ± standard error of the mean. A P-value lower than 0.05 is considered statistically significant.

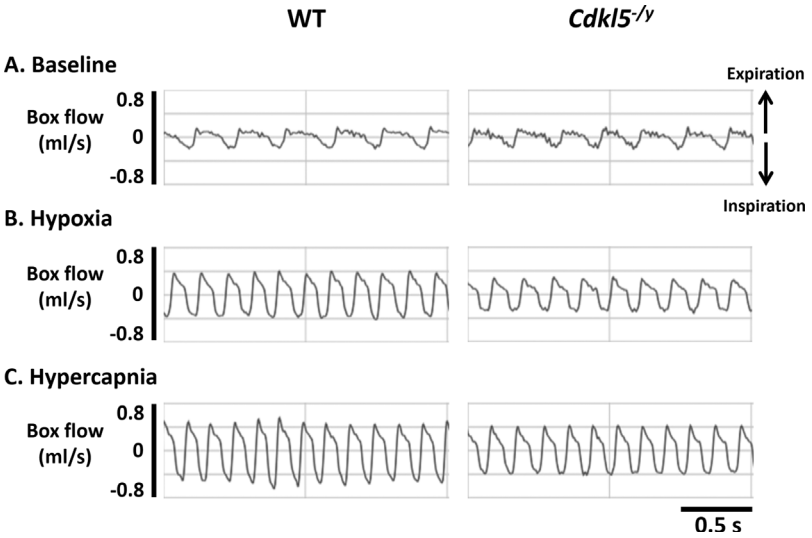

Fig. 1. Representative examples of the respiratory airflow of WT and *Cdkl5*$^{-/y}$ mice. Respiratory airflow of male mice at 1 month of age was measured using the whole body plethysmography during the baseline (A), hypoxia (B) and hypercapnia (C). Respiratory frequency is similar between WT and *Cdkl5*$^{-/y}$ mice; however, the respiratory airflow was lower in *Cdkl5*$^{-/y}$ mice during hypoxia and hypercapnia.

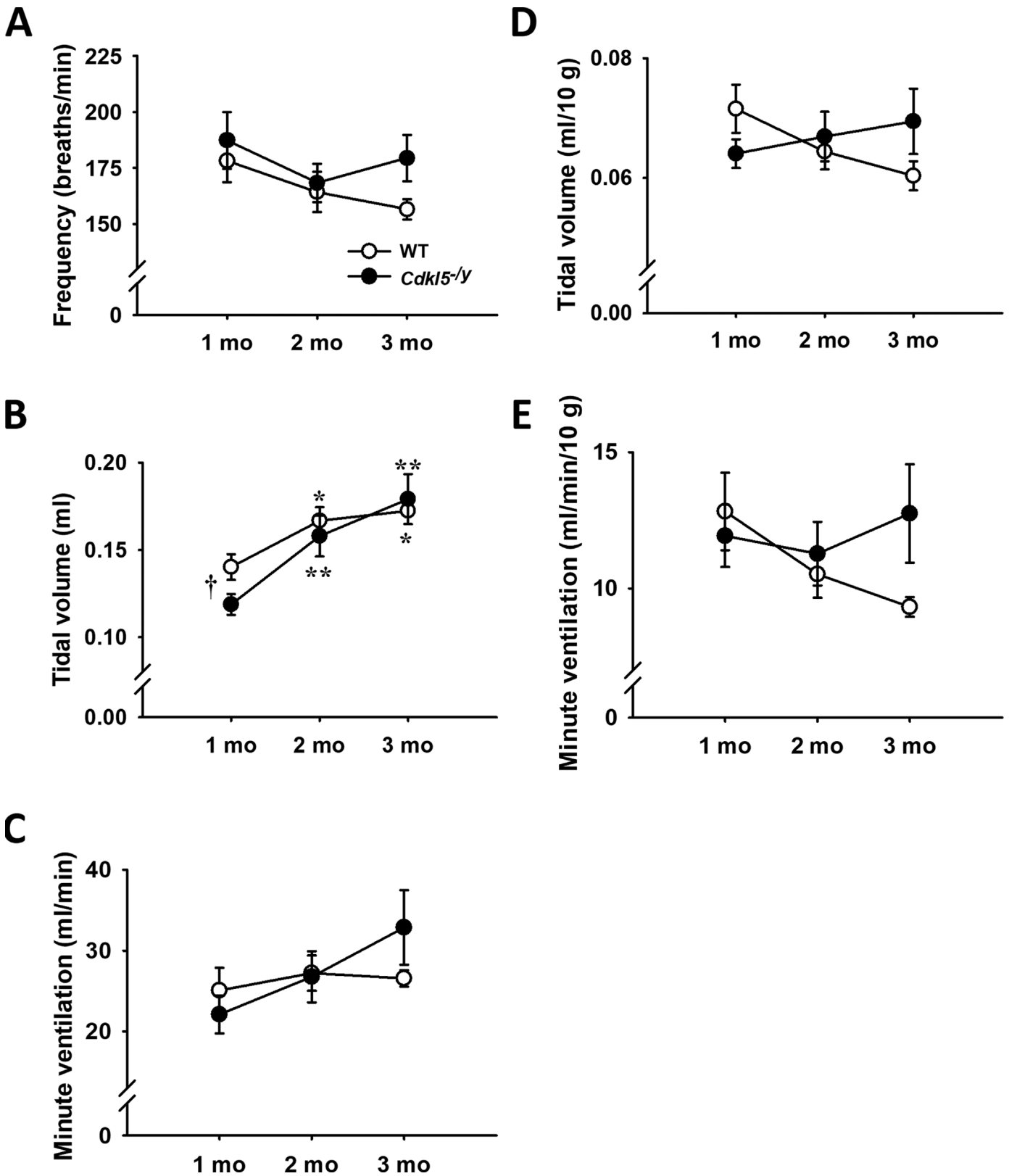

Fig. 2. The baseline breathing pattern in WT and *Cdkl5*$^{-/y}$ mice. The average respiratory frequency (A), tidal volume (B) and minute ventilation (C) during the baseline were measured from male mice at 1, 2 and 3 month of age. The tidal volume is significantly increased in both groups at 2 and 3 month of age, but the respiratory frequency and minute ventilation was similar across different ages. Panel D and E represent the tidal volume and minute ventilation data normalized by the weight. *P < 0.05; **P < 0.01 vs. 1 month. †: P = 0.039 significant difference between WT and *Cdkl5*$^{-/y}$ mice using *t*-test.

3. Result

3.1. Body weight

To examine whether loss of CDKL5 affects the body weight, the mice were weighed before measuring the respiratory behaviors. The body weight of *Cdkl5*$^{-/y}$ mice was comparable to that of WT mice at 1 month of age (18.5 ± 0.5 g in *Cdkl5*$^{-/y}$ vs. 19.7 ± 0.6 g in WT; P = 0.703), but significantly lower than WT controls at 3 months (P < 0.05, Table 1). In addition, we found significant weight gain with age in both genotypes (P < 0.05 compared to 1 month of age, Table 1).

3.2. Baseline respiratory behavior

Representative examples of the respiratory airflow of WT and *Cdkl5*$^{-/y}$ mice during baseline, hypoxia and hypercapnia are shown in Fig. 1. At 1 month of age, the baseline respiratory frequency of *Cdkl5*$^{-/y}$ mice was similar to that of WT mice (187 ± 13 breaths/min in *Cdkl5*$^{-/y}$ mice vs. 178 ± 10 breaths/min in WT) (Fig. 2A and Fig. 3A1). However, the tidal volume in *Cdkl5*$^{-/y}$ mice was slightly lower than that in WT mice (0.12 ± 0.01 ml in Cdkl5$^{-/y}$ vs. 0.14 ± 0.01 ml in WT, P = 0.039 by *t*-test), although this difference did not reach a significant level when using a two-way mixed design ANOVA (P = 0.112)(Fig. 2B). In addition, the minute ventilation of *Cdkl5*$^{-/y}$ mice was comparable to that of WT mice (Fig. 2C).

While respiratory frequency was not significantly influenced by age, the tidal volume of both WT mice and *Cdkl5*$^{-/y}$ mice gradually increased at 2 and 3 months of age. The tidal volume of WT mice significantly increased from 0.14 ± 0.01 ml at 1 month of age to 0.17 ± 0.01 ml at 2 and 3 months of age (P < 0.05, Fig. 2B). Similarly, *Cdkl5*$^{-/y}$ mice also exhibited increased tidal volume during the adult stage. Specifically, the tidal volume increased from 0.12 ± 0.01 ml at 1 month to 0.16 ± 0.01 ml at 2 months and 0.18 ± 0.01 ml at 3 months (P < 0.05, Fig. 2B). Notably, when the tidal volume and minute ventilation were normalized by the body weight, there were no significant differences between WT mice and *Cdkl5*$^{-/y}$ mice at all ages (P > 0.05, Figs. 2D–E), suggesting that the changes in the tidal volume of these mice are in proportion to the changes of their body weight with age.

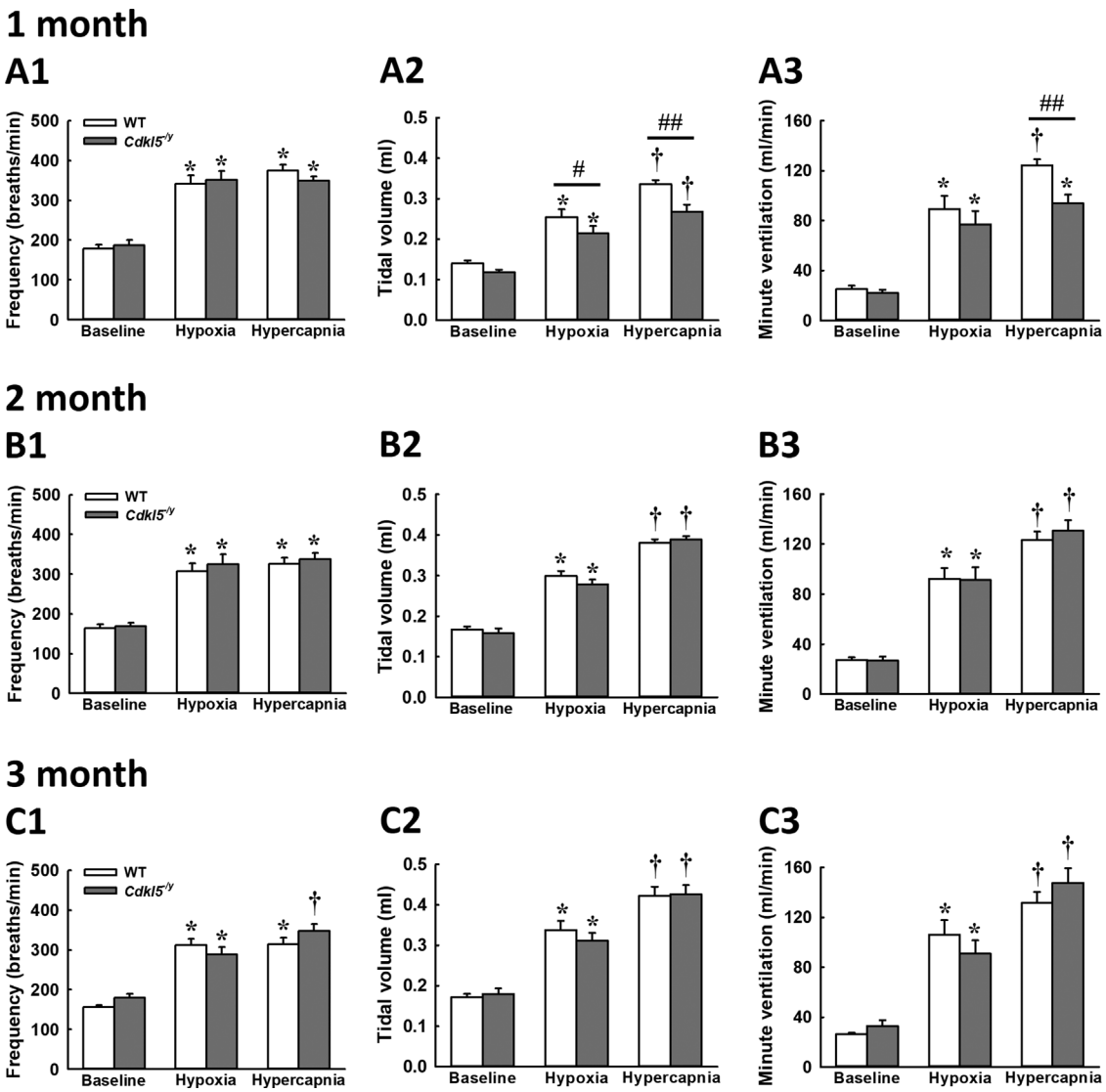

Fig. 3. The breathing pattern of WT and *Cdkl5*$^{-/y}$ mice in response to respiratory challenges. The respiratory behavior of *Cdkl5*$^{-/y}$ mice and their WT littermate controls were measured during the baseline, hypoxia and hypercapnia at one (A1–A3), two (B1–B3) and three (C1–C3) months of age. Both hypoxia and hypercapnia induced a significant increase in respiratory frequency, tidal volume and minute ventilation in both groups at all-time points. The tidal volume of *Cdkl5*$^{-/y}$ mice was significantly lower than that of WT mice at one but not two and three months of age during respiratory challenge. * P < 0.01 vs. baseline. † P < 0.05 vs. baseline and hypoxia. # P < 0.05; ## P < 0.01 significant difference between WT and *Cdkl5*$^{-/y}$ mice.

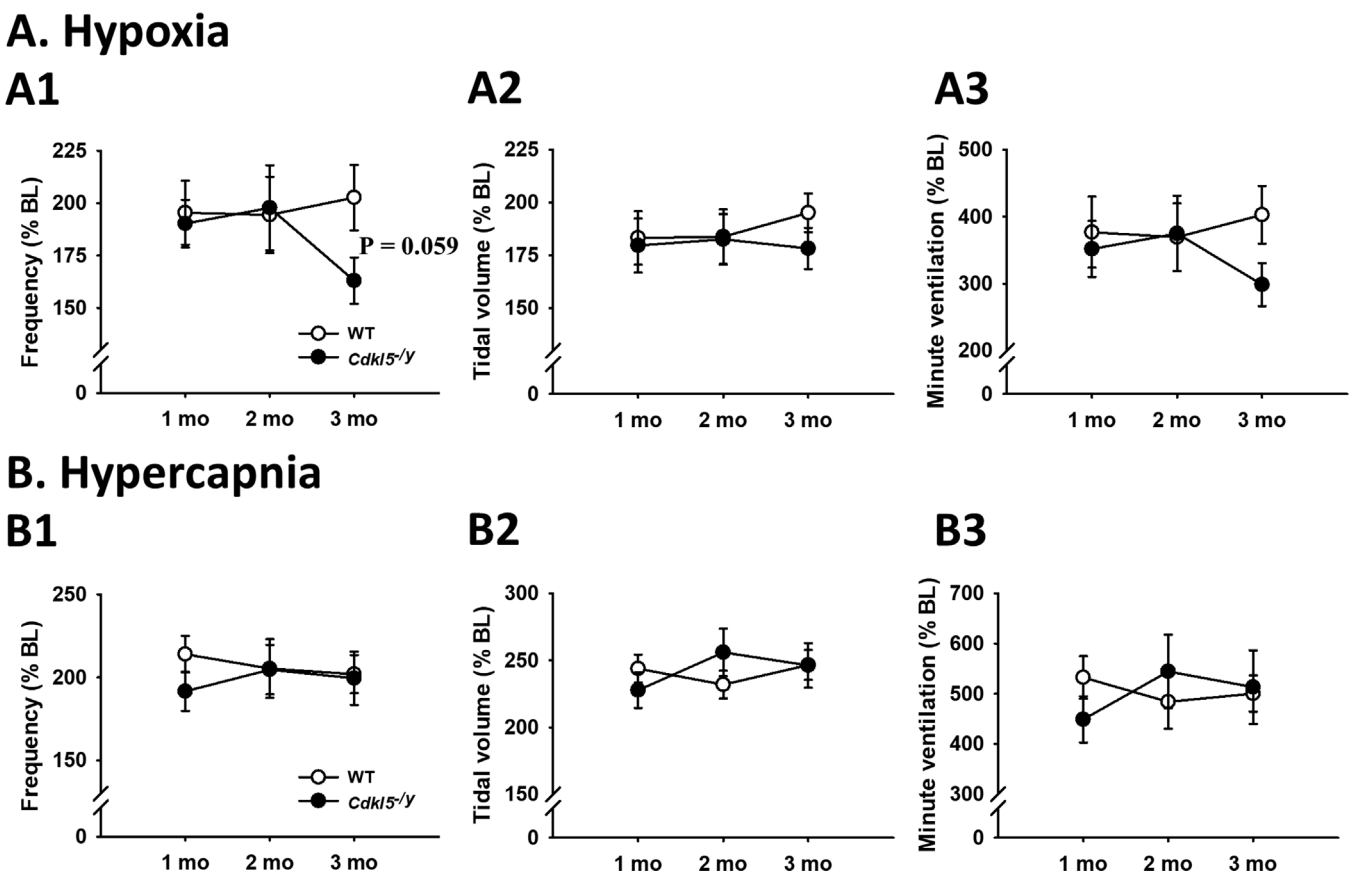

Fig. 4. The hypoxic and hypercapnic respiratory response of WT and *Cdkl5*$^{-/y}$ mice. The respiratory frequency, tidal volume and minute ventilation during hypoxia (A) or hypercapnia (B) were presented as a percentage of the baseline (% BL) at 1, 2 and 3 month of age. Hypoxia tend to induce less increase of respiratory frequency in *Cdkl5*$^{-/y}$ mice at 3 month of age compared to their littermate WT controls (P = 0.059, student-t test).

3.3. Respiratory behavior in response to challenge

During hypoxia and hypercapnia, both respiratory frequency and tidal volume significantly increased in WT mice and *Cdkl5*$^{-/y}$ mice, as expected (P < 0.01, Fig. 3). At one month of age, both hypoxic and hypercapnic challenge induced a similar increase of respiratory frequency in *Cdkl5*$^{-/y}$ mice (hypoxia: 351 ± 22 breaths/min; hypercapnia: 349 ± 11 breaths/min) and WT mice (hypoxia: 341 ± 21 breaths/min; hypercapnia: 375 ± 15 breaths/min)(Fig. 3A1). Both hypoxia and hypercapnia significantly elevated the tidal volume in *Cdkl5*$^{-/y}$ mice and WT mice (P < 0.01, Fig. 3A2), while the tidal volume was significantly reduced in *Cdkl5*$^{-/y}$ mice compared to WT controls (P < 0.05, Fig. 3A2). Specifically, the tidal volume was increased to 0.25 ± 0.02 ml and 0.34 ± 0.01 ml in WT mice during hypoxia and hypercapnia, respectively; while it was increased for less extent to 0.21 ± 0.02 ml during hypoxia and 0.27 ± 0.02 ml during hypercapniain in *Cdkl5*$^{-/y}$ mice. The reduced tidal volume response in *Cdkl5*$^{-/y}$ mice during hypercapnic challenge was correlated to a significant decrease in minute ventilation (94 ± 7 ml/min) comparing to that in WT mice (124 ± 5 ml/min) (P < 0.01, Fig. 3A3).

Notably, the deficient tidal volume shown in *Cdkl5*$^{-/y}$ mice during the juvenile stage (i.e., 1 month of age) was not observed in these mutants at 2 and 3 months of age. *Cdkl5*$^{-/y}$ mice exhibited similar tidal volume to WT mice upon respiratory challenge at 2 and 3 months age (Fig. 3B-C).

When we normalize the changes with the baseline respiration, we found that the percentage of increase in respiratory parameters during hypoxia and hypercapnia was similar between WT and *Cdkl5*$^{-/y}$ mice at all ages (Fig. 4). However, the hypoxic frequency response tended to be lower in *Cdkl5*$^{-/y}$ mice at 3 month of age (Fig. 4A1, P = 0.059 using *t*-test), and this trend was not observed when the animal received hypercapnic challenge (Fig. 4B1).

3.4. Sigh

The representative respiratory airflow generated during sigh was presented in Fig. 5A. Both WT and *Cdkl5*$^{-/y}$ mice exhibited sigh during normoxic breathing condition. The sigh frequency was slightly higher in *Cdkl5*$^{-/y}$ mice than WT mice, but this difference was merely close to a significant level (P = 0.067, Fig. 5B1). The inspiratory and expiratory duration of sigh were similar between groups (Figs. 5B2-5B3); however, the sigh tidal volume was significantly lower in *Cdkl5*$^{-/y}$ mice at 1 and 2 month of age (P < 0.05, Fig. C1). Although peak inspiratory flow was comparable between groups, the peak expiratory flow during sigh was significantly lower in *Cdkl5*$^{-/y}$ mice than WT mice from 1 to 3 month of age (P < 0.05, Fig. 5C3).

4. Discussion

This is the first report to investigate the role of CDKL5 in the respiratory function during normal, sigh and hypoxic/hypercapnic breathing at different developmental age. The present results demonstrate that respiratory frequency is similar between *Cdkl5*$^{-/y}$ and WT mice during resting normoxic breathing and respiratory challenge from 1 to 3 months of age, suggesting that the central control of respiratory rhythm in *Cdkl5*$^{-/y}$ mice may be unaffected during normal and high respiratory drives. However, a recent report showed that adult *Cdkl5* knockout mice (~4 months) have a higher occurrence of sleep apnea (Lo Martire et al., 2017), indicating that the respiratory rhythm may be differentially regulated by CDKL5 during sleep-wake cycle.

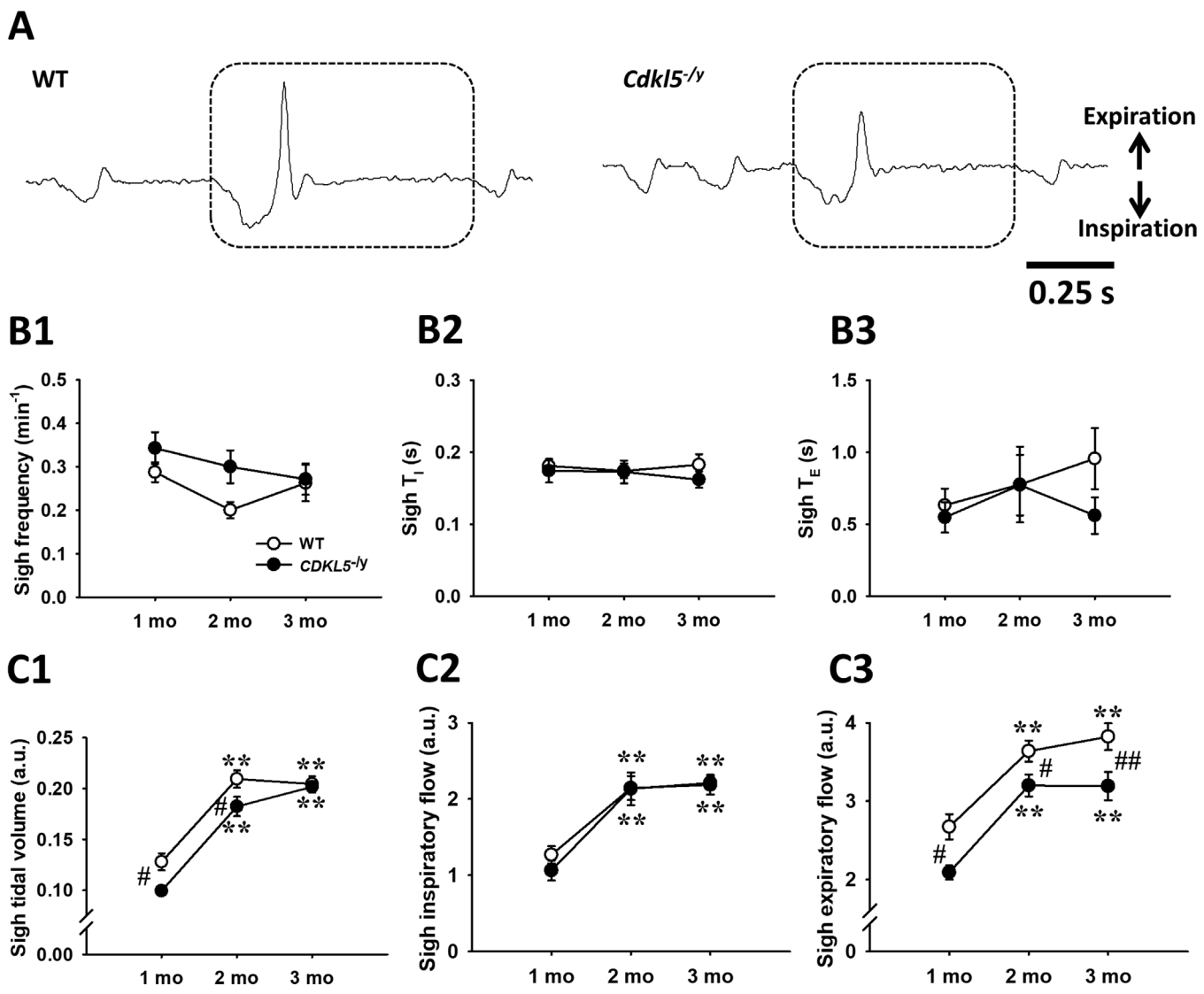

Fig. 5. Sigh breathing pattern in WT and *Cdkl5*$^{-/y}$ mice. Panel A presents the representative example of respiratory airflow during sigh (dash-line box) in a WT and *Cdkl5*$^{-/y}$ mice at 1 month of age. Panel B and C shows the averaged timing and amplitude parameter of sigh pattern, respectively. The frequency (B1), inspiratory duration (T_I, B2) and expiratory duration (T_E, B3) was similar between groups at all ages. However, both sigh tidal volume (C1) and peak expiratory flow (C3) were significantly lower in *Cdkl5*$^{-/y}$ mice. * P < 0.05; ** P < 0.01 vs. 1 mo. # P < 0.05; ## P < 0.01 significant difference between WT and *Cdkl5*$^{-/y}$ mice. mo: month; a.u.: arbitrary unit.

Our current study shows that *Cdkl5*$^{-/y}$ mice exhibit significant reduction in tidal volume during hypoxic/hypercapnic breathing and sigh at 1 and 2 month of age. Tidal volume is generated by the contraction of the respiratory pump muscles (e.g., diaphragm and intercostal muscles), which receive inputs from the cervical and thoracic spinal respiratory motoneurons (Lane, 2011; Lee and Fuller, 2011). Recent reports have demonstrated that the CDKL5 mutation is associated with impairment of excitatory and inhibitory synaptic integration and dendritic spine stability (Della Sala et al., 2016; Livide et al., 2015; Sivilia et al., 2016). Accordingly, we speculate that the reduced tidal volume could be due to alterations to the excitability of spinal respiratory motoneurons and/or an imbalance between excitatory/inhibitory synaptic inputs to the respiratory motoneurons. Moreover, the neuromuscular junctions and/or the contraction properties of respiratory muscles may be impaired in *Cdkl5*$^{-/y}$ mice. In addition to the alteration of motor component in *Cdkl5*$^{-/y}$ mice, the reduced tidal volume during respiratory challenge could be also due to blunted peripheral/central chemoreceptor sensitivity and/or impairment in respiratory integrated regions at the brainstem (i.e., raphe, nucleus of the solitary tract, ventral lateral medulla). The reduced tidal volume may unfavorably impact the alveolar oxygenation of *Cdkl5*$^{-/y}$ mice during the juvenile stage.

The tidal volume of normal breathing and sigh pattern of *Cdkl5*$^{-/y}$ mice recovers to normal levels at 3 months of age, suggesting the existence of a compensatory mechanism for recovering tidal volume. The main function of the respiration is to acquire oxygen and remove carbon dioxide, therefore, alterations of oxygen consumption and carbon dioxide production can also adjust the respiratory function. That is, changes in metabolism may lead to alteration of the ventilatory response in *Cdkl5*$^{-/y}$ mice. Several lines of evidence support that metabolic function may be modulated by CDKL5. Firstly, the body weight of *Cdkl5*$^{-/y}$ mice was significantly lower than that of WT mice at 3 months of age in the present study (Table 1), reflecting that the metabolic rate is likely increased in *Cdkl5*$^{-/y}$ mice. Secondly, previous studies reported that *Cdkl5*$^{-/y}$ mice exhibit enhanced locomotor activity compared to WT mice (Jhang et al., 2017; Wang et al., 2012), suggesting that oxygen demand could be increased in *Cdkl5*$^{-/y}$ mice. Finally, a clinical study found that patients with CDKL5 deletions had an increased level of thyroid hormone (e.g., free-T4) which is usually associated with augmented metabolism (Stagi et al., 2015). These results suggest that changes of ventilatory behavior in adult *Cdkl5*$^{-/y}$ mice may potentially result from metabolic alterations.

The inspiratory tidal volume during the normal breathing, sigh and

respiratory challenge recovered during the adult stage; however, the peak expiratory flow during sigh remained attenuated at 3 month of age. This finding suggested that CDKL5 may have distinct regulatory roles on inspiratory and expiratory function. The impairment of expiratory airflow may be caused by long-term dysfunction of several respiratory components, including expiratory neural circuit, abdominal muscle and/or upper airway muscle. This abnormality of sigh pattern was also observed in the mouse model of Rett syndrome (*Mecp2*$^{-/y}$ mice)(Voituron et al., 2010). Mari et al. (2005) demonstrated that CDKL5 is co-expressed with MeCP2 in the mouse central nervous system (Mari et al., 2005), and some reports indicated that CDKL5 and MeCP2 can interact each other and may belong to the similar molecular pathway (Carouge et al., 2010; Mari et al., 2005). Although *CDKL5* mutation has been previously considered as a factor contributing to atypical Rett syndrome (Hagebeuk et al., 2013; Mari et al., 2005; Scala et al., 2005), a recent concept suggested CDKL5 disorder should be considered as an independent entity (Mangatt et al., 2016). Accordingly, future studies are warranty to comprehensively evaluate the functional role of CDKL5 on respiratory system and compare respiratory phenotypes of *Mecp2*$^{-/y}$ mice versus *CDKL5*$^{-/y}$ mice (Jiang et al., 2017; Ramirez et al., 2013).

In accordance with the clinical case report that children with *CDKL5* mutations exhibit respiratory abnormalities (Hagebeuk et al., 2013), our current study demonstrated that both normal breathing and sigh pattern are significantly altered in *Cdkl5*$^{-/y}$ mice at first 3 months after birth, supporting that CDKL5 regulates respiratory function, at least during the juvenile and early adult stage. Ventilation is a critical physiological function that maintains blood O_2 and CO_2 levels, metabolism and acid-base homeostasis. Impairment of ventilation during the early postnatal stage may lead to certain pathological consequences and alter normal development. Therefore, therapeutics or interventions to improve breathing function during early age could be important for patients with CDKL5 disorder.

Grants

This work was supported by the Ministry of Science and Technology of Taiwan [MOST105-2320-B-004-001 (WL) and 105-2628-B-110-002-MY3 (KZL)], NSYSU-KMU Joint Research Project (106-I009) (KZL) and International Foundation of CDKL5 Research, USA (WL).

References

Archer, H.L., Evans, J., Edwards, S., Colley, J., Newbury-Ecob, R., O'Callaghan, F., Huyton, M., O'Regan, M., Tolmie, J., Sampson, J., Clarke, A., Osborne, J., 2006. CDKL5 mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. J. Med. Genet. 43, 729–734.

Carouge, D., Host, L., Aunis, D., Zwiller, J., Anglard, P., 2010. CDKL5 is a brain MeCP2 target gene regulated by DNA methylation. Neurobiol. Dis. 38, 414–424.

Chen, Q., Zhu, Y.C., Yu, J., Miao, S., Zheng, J., Xu, L., Zhou, Y., Li, D., Zhang, C., Tao, J., Xiong, Z.Q., 2010. CDKL5, a protein associated with rett syndrome, regulates neuronal morphogenesis via Rac1 signaling. J. Neurosci. 30, 12777–12786.

Della Sala, G., Putignano, E., Chelini, G., Melani, R., Calcagno, E., Michele Ratto, G., Amendola, E., Gross, C.T., Giustetto, M., Pizzorusso, T., 2016. Dendritic spine instability in a mouse model of CDKL5 disorder is rescued by insulin-like growth factor 1. Biol. Psychiatry 80, 302–311.

Hagebeuk, E.E., van den Bossche, R.A., de Weerd, A.W., 2013. Respiratory and sleep disorders in female children with atypical Rett syndrome caused by mutations in the CDKL5 gene. Dev. Med. Child Neurol. 55, 480–484.

Jhang, C.L., Huang, T.N., Hsueh, Y.P., Liao, W., 2017. Mice lacking cyclin-dependent kinase-like 5 manifest autistic and ADHD-like behaviors. Human Mol. Genet. 26, 3922–3934.

Jiang, C., Cui, N., Zhong, W., Johnson, C.M., Wu, Y., 2017. Breathing abnormalities in animal models of Rett syndrome a female neurogenetic disorder. Respir. Physiol. Neurobiol. 245, 45–52.

Kao, F.C., Su, S.H., Carlson, G.C., Liao, W., 2015. MeCP2-mediated alterations of striatal features accompany psychomotor deficits in a mouse model of Rett syndrome. Brain Struct. Funct. 220, 419–434.

Kobayashi, Y., Tohyama, J., Kato, M., Akasaka, N., Magara, S., Kawashima, H., Ohashi, T., Shiraishi, H., Nakashima, M., Saitsu, H., Matsumoto, N., 2016. High prevalence of genetic alterations in early-onset epileptic encephalopathies associated with infantile movement disorders. Brain Dev. 38, 285–292.

Lane, M.A., 2011. Spinal respiratory motoneurons and interneurons. Respir. Physiol. Neurobiol. 179, 3–13.

Lee, K.Z., Fuller, D.D., 2011. Neural control of phrenic motoneuron discharge. Respir. Physiol. Neurobiol. 179, 71–79.

Lee, K.Z., Qiu, K., Sandhu, M.S., Elmallah, M.K., Falk, D.J., Lane, M.A., Reier, P.J., Byrne, B.J., Fuller, D.D., 2011. Hypoglossal neuropathology and respiratory activity in pompe mice. Front. Physiol. 2, 31.

Lee, K.Z., Huang, Y.J., Tsai, I.L., 2014. 2014: Respiratory motor outputs following unilateral midcervical spinal cord injury in the adult rat. J. Appl. Physiol. 116 (1985), 395–405.

Lee, K.Z., Chiang, S.C., Li, Y.J., 2017. Mild acute intermittent hypoxia improves respiratory function in unanesthetized rats with midcervical contusion. Neurorehabil. Neural Repair 31, 364–375.

Livide, G., Patriarchi, T., Amenduni, M., Amabile, S., Yasui, D., Calcagno, E., Lo Rizzo, C., De Falco, G., Ulivieri, C., Ariani, F., Mari, F., Mencarelli, M.A., Hell, J.W., Renieri, A., Meloni, I., 2015. GluD1 is a common altered player in neuronal differentiation from both MECP2-mutated and CDKL5-mutated iPS cells. Eur. J. Human Genet.: EJHG 23, 195–201.

Lo Martire, V., Alvente, S., Bastianini, S., Berteotti, C., Silvani, A., Valli, A., Viggiano, R., Ciani, E., Zoccoli, G., 2017. CDKL5 deficiency entails sleep apneas in mice. J. Sleep Res. 26, 495–497.

Mangatt, M., Wong, K., Anderson, B., Epstein, A., Hodgetts, S., Leonard, H., Downs, J., 2016. Prevalence and onset of comorbidities in the CDKL5 disorder differ from Rett syndrome. Orphanet J. Rare Dis. 11, 39.

Mari, F., Azimonti, S., Bertani, I., Bolognese, F., Colombo, E., Caselli, R., Scala, E., Longo, I., Grosso, S., Pescucci, C., Ariani, F., Hayek, G., Balestri, P., Bergo, A., Badaracco, G., Zappella, M., Broccoli, V., Renieri, A., Kilstrup-Nielsen, C., Landsberger, N., 2005. CDKL5 belongs to the same molecular pathway of MeCP2 and it is responsible for the early-onset seizure variant of Rett syndrome. Human Mol. Genet. 14, 1935–1946.

Montini, E., Andolfi, G., Caruso, A., Buchner, G., Walpole, S.M., Mariani, M., Consalez, G., Trump, D., Ballabio, A., Franco, B., 1998. Identification and characterization of a novel serine-threonine kinase gene from the Xp22 region. Genomics 51, 427–433.

Nichols, N.L., Vinit, S., Bauernschmidt, L., Mitchell, G.S., 2015. Respiratory function after selective respiratory motor neuron death from intrapleural CTB-saporin injections. Exp. Neurol. 267, 18–29.

Ramirez, J.M., Ward, C.S., Neul, J.L., 2013. Breathing challenges in Rett syndrome: lessons learned from humans and animal models. Respir. Physiol. Neurobiol. 189, 280–287.

Ricciardi, S., Ungaro, F., Hambrock, M., Rademacher, N., Stefanelli, G., Brambilla, D., Sessa, A., Magagnotti, C., Bachi, A., Giarda, E., Verpelli, C., Kilstrup-Nielsen, C., Sala, C., Kalscheuer, V.M., Broccoli, V., 2012. CDKL5 ensures excitatory synapse stability by reinforcing NGL-1-PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. Nat. Cell Biol. 14, 911–923.

Rusconi, L., Salvatoni, L., Giudici, L., Bertani, I., Kilstrup-Nielsen, C., Broccoli, V., Landsberger, N., 2008. CDKL5 expression is modulated during neuronal development and its subcellular distribution is tightly regulated by the C-terminal tail. J. Biol. Chem. 283, 30101–30111.

Scala, E., Ariani, F., Mari, F., Caselli, R., Pescucci, C., Longo, I., Meloni, I., Giachino, D., Bruttini, M., Hayek, G., Zappella, M., Renieri, A., 2005. CDKL5/STK9 is mutated in Rett syndrome variant with infantile spasms. J. Med. Genet. 42, 103–107.

Sivilia, S., Mangano, C., Beggiato, S., Giuliani, A., Torricella, R., Baldassarro, V.A., Fernandez, M., Lorenzini, L., Giardino, L., Borelli, A.C., Ferraro, L., Calza, L., 2016. CDKL5 knockout leads to altered inhibitory transmission in the cerebellum of adult mice. Genes Brain Behav. 15, 491–502.

Stagi, S., Cavalli, L., Congiu, L., Scusa, M.F., Ferlini, A., Bigoni, S., Benincasa, A., Rossi, B., Pini, G., 2015. Thyroid function in Rett syndrome. Horm. Res. Paediatr. 83, 118–125.

Voituron, N., Zanella, S., Menuet, C., Lajard, A.M., Dutschmann, M., Hilaire, G., 2010. Early abnormalities of post-sigh breathing in a mouse model of Rett syndrome. Respir. Physiol. Neurobiol. 170, 173–182.

Wang, I.T., Allen, M., Goffin, D., Zhu, X., Fairless, A.H., Brodkin, E.S., Siegel, S.J., Marsh, E.D., Blendy, J.A., Zhou, Z., 2012. Loss of CDKL5 disrupts kinome profile and event-related potentials leading to autistic-like phenotypes in mice. Proc. Natl. Acad. Sci. U. S. A. 109, 21516–21521.