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# Loss of CDKL5 disrupts respiratory function in mice

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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

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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.

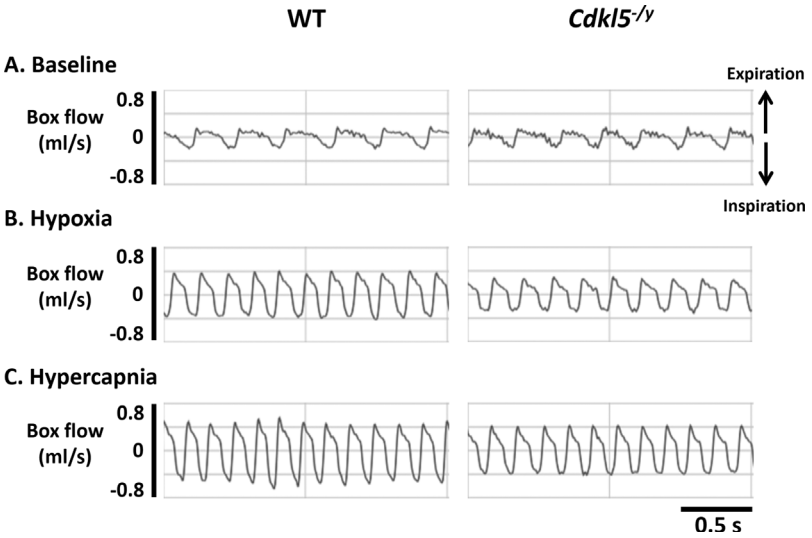


**Fig. 1.** Representative examples of the respiratory airflow of WT and *Cdkl5*$^{-/y}$ mice. Respiratory airflow of male mice at 1 month of age was measured using the whole body plethysmography during the baseline (A), hypoxia (B) and hypercapnia (C). Respiratory frequency is similar between WT and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