Rett syndrome: Neurologic and metabolic aspects
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Respiratory and sleep disorders in girls with atypical Rett syndrome caused by CDKL5 mutations

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Abstract

Aim: In female children with drug-resistant seizures and developmental delay from birth, atypical Rett syndrome caused by mutation in the CDKL5 gene should be considered. Several clinical features resemble classic Rett syndrome. Respiratory and sleep abnormalities are frequently present in Rett syndrome, whereas little is known in patients with CDKL5 mutations.

Methods: In four genetically confirmed female patients with CDKL5 (age range 2-15y), the presence of breathing and sleep abnormalities was evaluated using the validated Sleep Disturbance Scale for Children and polysomnography (PSG).

Results: The Sleep Disturbance Scale for Children indicated disorders of initiating and maintaining sleep, daytime somnolence and sleep breathing disorders. In one patient, PSG showed central apneas during sleep: her total Apnea Hypopnea Index (AHI) was 4.9, of which the central AHI was 3.4/h. When awake, central apneas were present in 2 of the 4 girls (central AHI 28 and 41/h respectively), all preceded by hyperventilation. PSG showed low rapid eye movement (REM) sleep (9.7-18.3%), frequent awakenings, and low sleep efficiency (range 59-78%).

Interpretation: Episodic hyperventilation followed by central apneas were present while awake in 2 of 4 patients. This may indicate failure of brainstem respiratory centers. In addition, low REM sleep, frequent arousals (not caused by apneas/seizures) and low sleep efficiency were present. Similar to Rett syndrome, in patients with CDKL5 mutations, PSG seems warranted to evaluate breathing and sleep disturbances.

What the paper adds:
- Breathing disturbances are present in CDKL5 patients
- Polysomnography (PSG) showed central apneas when awake
- Sleep disturbances are present in CDKL5 patients
- PSG revealed low Rem sleep, frequent awakenings and low sleep efficiency
- As in the classic Rett syndrome, PSG is also warranted in CDKL5 patients

Keywords: CDKL5 syndrome, Rett syndrome, respiratory disturbances, central apnea, sleep disturbances, Sleep Disturbance Scale for Children
Introduction

Rett syndrome is a genetic neurodevelopmental disorder that mainly affects female children. The clinical diagnosis is based on the presence of regression in combination with the loss of purposeful hand skills, motor and communication function, cognitive impairment, the development of stereotypic hand function and seizures. In addition, breathing disturbances such as hypoventilation, central apnea, episodic hyperventilation and breath holding may be present. They occur mainly when awake, and can be life threatening. In 97% of patients with Rett syndrome a dominant mutation in the gene encoding methyl-CpG binding protein 2 (MECP2) on the X-chromosome is found, which enhances or activates gene expression.

In female children with drug-resistant seizures in the first year of life and severe psychomotor delay from birth, atypical Rett syndrome caused by a mutation in the cyclin-dependent kinase-like 5 (CDKL5) gene should be considered. Clinically the patients resemble those with classic Rett syndrome, and they have impairment of purposeful hand function, cannot sit without support or walk, lack expressive language, and demonstrate autistic-like features. Neurological examination reveals trunk hypotonia and deceleration of head growth. In contrast to classic Rett syndrome, female children with CDKL5 mutations experience no regression and the delay of psychomotor development is present from birth onwards. Furthermore, in female children with CDKL5 mutations frequent daily epileptic seizures develop in the first 2 months of life, in contrast with classic Rett syndrome in which seizures develop after 2 years of age. Seizures consist of generalized tonic seizures with flushing of the face and sometimes tonic seizures followed by a clonic phase. Epileptic encephalopathy with infantile spasms and hypsarrhythmia often develop. Seizures may diminish over time, but often daily tonic, myoclonic and atypical absence seizures persist.

Recently, a direct interaction was demonstrated between MECP2 and the CDKL5 gene, i.e. CDKL5 is a novel MECP2 repressed target gene. Therefore, it is likely that clinical symptoms overlap and that the well-known respiratory and sleep disturbances in MECP2 patients, are also present in those with CDKL5 mutations. However, because no data are available, this study examines the presence of breathing and sleep abnormalities in a small series of patients with CDKL5 mutations.
Patients and methods

We investigated 4 female children with CDKL5 mutations attending the Sleep Center of SEIN (Stichting Epilepsie Instellingen Nederland) because of sleep disturbances. As the protocol was similar to that used in all cases with clinical suspicion of a sleep disorder, it was not necessary to consult the medical ethical committee. Parents gave written informed consent for participation as well as permission to publish the results.

All 4 patients had a severe developmental delay and experienced seizures. The validated Sleep Disturbance Scale for Children (SDSC, Dutch version), a parental questionnaire, was used to evaluate sleep and night-time breathing disorders. Questions are answered on a Likert-type scale, with higher values reflecting greater clinical severity: 1=never, 2=occasionally, 3=sometimes, 4=often (3-5 times a week), and 5=always. The maximum score for all questions was 100 with a total score of ≥ 70 considered clinically relevant.

Polysomnography (PSG) recordings were performed overnight, and during a few hours in the awake state during the day, using the guidelines for recording of sleep/snoring in children of the American Association of Sleep Medicine. The following measurements were evaluated: snoring, air flow (thermistor and nasal pressure) and respiratory effort, oxygen saturation (SpO₂), heart rate frequency, transcutaneous partial pressure of carbon dioxide (pCO₂; 2 patients). Video-electroencephalography (EEG), electro-oculography (EOG) and electromyography (EMG) provided data for the assessment of sleep, epileptic discharges and indicated the presence of seizures. Apneas were defined as a 90% reduction of the amplitude of air flow with a duration of at least 2 breaths. In a central apnea there was no airflow and no abdominal-thoracic movement combined with at least 3% desaturation, or it was defined as an apnea lasting at least 20 seconds. An obstructive apnea was defined as no airflow, despite chest wall and abdominal movements. In mixed central-obstructive apnea both phenomena were present. A hypopnea was defined as a 50% reduction of breathing amplitude for at least 2 breaths combined with a decrease of 3% in SpO₂. The apnea-hypopnea index (AHI) is the mean total number of apneas and hypopneas per hour of sleep. An AHI of 1 or more is regarded as pathological. Based on our normal values this threshold is 3/hour.

Breathing disorders in patient with classic Rett often occur in the awake state. A central apnea index (cAHI) is calculated while wake (central apneas/total recording time minus total sleep time).
SpO\textsubscript{2} was continuously measured using pulse oximetry. Transcutaneous pCO\textsubscript{2} was considered to be abnormal when > 50 and < 35 mmHg. Sleep efficiency (total sleep time/time lights off x 100), percentage of sleep stages, sleep onset latency (duration from light off to sleep onset), and number of awakenings were evaluated.

**Results**

The 4 patients with *CDKL5* mutations had a mean age of 6 years 6 months (range 2-15 y). Their weight was average or below average (range -2 SD to 0 SD) whereas their height was far below average for age (range -3 SD to -1 SD). The body mass index (BMI) was normal in 3 of the children, whereas the eldest patient had a lower BMI of 13.3. Table 1 presents the patients’ clinical characteristics, genetic mutation and PSG data.

Neurological investigation showed severe psychomotor developmental delay. All the younger female children had cerebral visual impairment to some extent, such as low vision and diminished eye contact, intermittent nystagmus and squinting. In addition, they had profound trunk hypotonia, could not sit without support or walk, and had hand stereotypies only and no purposeful hand movements. The 15-year-old girl had a milder phenotype; she could walk, had some voluntary hand movements, good eye contact, used a single word and seizures were less frequent and severe. The younger female children had daily tonic seizures. All used multiple anti-epileptic drugs. Two of the children were on a ketogenic diet, besides medication for gastroesophageal reflux, oral laxatives and (patient 4) promethazine. Patient 2 used melatonin, not during the PSG recordings.
Table 1: Clinical characteristics and respiratory data.

<table>
<thead>
<tr>
<th>No</th>
<th>Age years</th>
<th>Mutation</th>
<th>AED</th>
<th>Seizures</th>
<th>AHI sleep</th>
<th>SpO₂ (lowest %) sleep</th>
<th>Central apnea total (awake)</th>
<th>Wake Hours</th>
<th>C-AHI apnea/awake</th>
<th>Central apnea mean sec (shortest-longest)</th>
<th>Snoring min (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>c.656A&gt;C</td>
<td>VGB, LEV, LTG, RFN</td>
<td>Yes, daily; t, m</td>
<td>4.9</td>
<td>96.0 (87)</td>
<td>358</td>
<td>8.7</td>
<td>41.0</td>
<td>7.1 (3.9-15.6)</td>
<td>73.5 (16.6)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>c.660_664dup</td>
<td>OXC, CLB, LEV, Ket</td>
<td>Yes, daily; t, cp</td>
<td>0.5</td>
<td>N.A.</td>
<td>3</td>
<td>6.7</td>
<td>0.4</td>
<td>8.3 (6.7-10.6)</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>c.283-3_290del</td>
<td>VPA, CBZ, CLB, Ket</td>
<td>Yes, daily; t</td>
<td>0.4</td>
<td>96.0 (92)</td>
<td>3</td>
<td>4.7</td>
<td>0.6</td>
<td>12.4 (7.6-17.7)</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>c.2635_2636del</td>
<td>VPA, CBZ</td>
<td>Yes, monthly; t</td>
<td>0.1</td>
<td>96.9 (93)</td>
<td>148</td>
<td>5.3</td>
<td>28.0</td>
<td>10.9 (5.2-38.1)</td>
<td>38.4 (6.7)</td>
</tr>
</tbody>
</table>

AED = anti-epileptic drugs: VGB = Vigabatrine, LEV = Levetiracetam, LTG = Lamotrigine, RFN = Rufinamide, OXC=oxcarbamazepine, CLB = Clobazam, CBZ = Carbamazepine, VPA = Valproic acid, Ket = ketogenic diet
T = tonic seizures, m = myoclonic seizures, cp = complex partial seizures
AHI = apnea hypopnea index during sleep; C-AHI: central apnea hypopnea index in the awake state (total amount of apneas/ time awake)
SpO₂ = mean oxygen saturation
N.A. = not available
<table>
<thead>
<tr>
<th>ID No.</th>
<th>Age years</th>
<th>Sleep onset (min)</th>
<th>Stage 2 (min) (%TST)</th>
<th>SWS (min) (%TST)</th>
<th>REM latency (min)</th>
<th>REM sleep (Min) (%TST)</th>
<th>Number of Awakenings</th>
<th>Sleep efficiency (%)</th>
<th>SDSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5.8</td>
<td>180.5 (40.4)</td>
<td>214.5 (48)</td>
<td>39.5</td>
<td>43.5 (9.7)</td>
<td>7</td>
<td>59.3</td>
<td>56 (DIMS 23, SWTD 12, DOES 8)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1.3</td>
<td>479.5 (86.7)</td>
<td>68.0 (12.3)</td>
<td>no REM</td>
<td>no REM</td>
<td>26</td>
<td>78</td>
<td>71 (DIMS 17, SWTD 22, DOES 17)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>133.3</td>
<td>209.0 (44)</td>
<td>167.0 (35.5)</td>
<td>150.5</td>
<td>81.0 (17.2)</td>
<td>10</td>
<td>62</td>
<td>60 (DIMS 18, SWTD 15, SBD 10)</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>14.8</td>
<td>315.5 (52.9)</td>
<td>117.5 (19.7)</td>
<td>136.5</td>
<td>109.0 (18.3)</td>
<td>52</td>
<td>83</td>
<td>38 (DIMS 14, SWTD 6, DOES 10)</td>
</tr>
</tbody>
</table>

Sleep onset = time before any sleep stage is reached after lights out
TST = total sleep time
SWS = slow wave sleep
REM = rapid eye movement sleep
SDSC = Sleep Disturbance Scale for Children, total score ≥ 70 significant (bold)
DIMS = disorders of initiating and maintaining sleep (maximum score 26, significant > 17)
SWTD = sleep wake transition disorders (maximum score > 21, significant > 14)
DOES = disorders of excessive somnolence (maximum score 20, significant > 13)
SBD = Sleep breathing disorders (maximum score 15, significant > 7)
The history of the patient revealed sleeping disturbances in all the children, consisting mainly of insomnia and frequent awakenings. The Sleep Disturbance Scale for Children questionnaire confirmed the presence of different sleep disorders (Patients 1-3; Table 2). Most parents reported disorders of initiating and maintaining sleep (DIMS), sleep wake transition disorders (SWTD), excessive daytime somnolence (DOES) and (in patient 3 only) sleep breathing disorders (SBD). In Patient 4 the SDSC scores were not (significantly) abnormal.

PSGs were started in the afternoon (while awake) until the following morning. The recording time in the sleep state ranged from 916-972 minutes and in the awake state from 447-471 minutes. PSG showed severely prolonged delayed sleep onset in one female child only (Patient 3; sleep onset 133 min). Remarkably, the percentage of REM sleep was low in all our patients with CDKL5 mutations (9.7-18.3%) and absent in one girl. In all patients the sleep efficiency was low (median range 59-78%), caused by frequent and long-lasting awakenings (median range 7-52 during the night). Although interictal epileptiform activity was abundant, these awakenings were not caused by seizures.

During sleep, only the youngest girl (Patient 1, AHI 4.9/h) had some obstructive apneas (oAHI 0.5/h) not clinically relevant, and some central apneas (cAHI 3.4/h). Some of these central apneas occurred during REM sleep and are considered physiological. In the awake state, central apneas were more prominently present in 2 of the 4 patients (cAHI 28/h and 41/h, respectively), and most frequent in the youngest child (Patient 1). The mean duration of these central apneas was longer in the oldest girl (Patient 4) with a mean of 10.9 seconds (compared to Patient 2 with a mean of 7.1 s); the longest central apnea in Patient 4 lasted for 38 seconds. The PSGs showed that a period of agitation, increase of hand stereotypies and hyperventilation preceded the apneas (Figure 1). Unfortunately transcutaneous pCO₂ measurements were successfully performed in only one child (Patient 2).
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Figure 1: A 3-minute polysomnographic recording at night, during the awake state. Eye movements (electro-oculogram [EOG]; channel 1), muscle activity on the chin (Electromyography [EMG]; channel 2), and video-electroencephalography (EEG; channel 3) confirm that this female child is awake. Simultaneous with stereotypic hand movements and agitation, central apnoeas (duration 7–16.23s) occur. 1, EOG (eye movements); 2, EMG at the chin; 3, EEG (six channels), owing to muscle artefacts, for better visualization three left-side channels are shown; 4, EMG for detecting movement of the legs; 5, Nasal pressure (nasal air flow [pressure measurements between inspiration and expiration]); 6, thermistor (air flow [temperature measurements between inspiration and expiration]); 7, Flow_Cu (computer calculation of signals 5, 6, 8 to detect flattening of airflow); 8 (top), thorax movements; 8 (bottom), abdominal movements; 9, pulse (heart frequency/min); 10, SpO\textsubscript{2} (oxygen saturation).
Discussion

In 2 of our 4 patients with CDKL5 mutations, respiratory disturbances (all of the central type) were present during the awake state and were preceded by frequent episodic hyperventilation (cAHI 28 and 41, respectively). Although formal PCO₂ measurements failed in 3 of the 4 patients, we postulate that these apneas are probably of the hypocapnic type and indicate failure of the respiratory feedback loop and central regulatory nuclei in the brainstem.

Since 2003, about 85 girls with atypical Rett syndrome and a CDKL5 gene mutation have been described, characterized by severe intellectual disability and developmental delay, intractable seizures, pronounced hypotonia and hand stereotypies.^4,5^ The presence of hyperventilation was mentioned only in one of the first clinical surveys.^5^ A recent study in 10 patients with CDKL5 mutations demonstrated the presence of cardiorespiratory dysrhythmias such as tachypnoea (11.7%), deep breathing (4%), and apneas (1.4%) and breath holding (3.25%).^9^ Figure 1 shows that in a time window of 3 minutes, 7 periods of central apneas occurred, all preceded by a period of hyperventilation. This is not compatible with breath holding as explanation of the phenomena in our patients.

In the classic Rett syndrome, PSG studies revealed the presence of hyperventilation in the awake state, often followed by prolonged apneas which result in hypoxemia.^10^ Three groups of daytime respiratory disturbances were identified: the forceful type (breath holding and air expulsion), feeble (superficial breathing) and apneustic breathers.^11^ Differentiating between these breathing abnormalities is important, since especially the feeble breathers do not tolerate opiates.^11^ Central apneas can be treated with acetazolamide^12^, nasal oxygen therapy^13^ and positive air pressure therapy.^14^

Periods of agitation and increase of hand stereotypies and (at the same time) episodic hyperventilation, preceded the occurrence of central apneas. The breathing disorder diminishes when the hands are fixated. Although behavioral factors might influence the occurrence of the breathing disorder, clinical studies in classic Rett syndrome have shown dysphagia and cardiorespiratory irregularities during the day and night, also suggesting brainstem dysfunction.^2^ Thus, there is evidence that brainstem dysfunction is also the pathogenic mechanism behind the respiratory disturbances.
In our 4 patients with *CDKL5* mutations, the parents reported sleep disturbances, mainly disorders of initiating and maintaining sleep and excessive daytime napping, but no report of nighttime emotional behavior, which is typical for the classic Rett syndrome.

In patients with classic Rett syndrome, sleep questionnaires revealed sleep problems in ≥ 80%. They consist of irregular sleep/wake patterns, excessive daytime sleep, and nighttime emotional behavior like screaming, crying and laughing. Sleep diaries (7 x 24 h), showed increased total nighttime sleep and excessive daytime sleep, but no decrease despite aging. PSG revealed a decreased REM sleep percentage. This was also found in all our patients with *CDKL5* mutations. We cannot explain this low percentage of REM sleep, since this is not typical for children with a developmental delay. In addition, frequent arousals (not caused by apneas or seizures) and low sleep efficiency were present. This is similar to classic Rett syndrome.

Epilepsy in patients with *CDKL5* mutations is often more severe than in those with classic Rett syndrome. Electroencephalography showed frequent epileptic discharges in all and sometimes tonic seizures. Having seizures is known to increase total sleep time and daytime sleep.

Some limitations of our study need discussing. Because the pCO$_2$ measurement failed we were unable to prove our hypothesis formally. Also, owing to the rarity of this disorder, we could evaluate only 4 patients with *CDKL5* mutations. Finally, the follow-up evaluation of these children is still in progress.

Patients with Rett syndrome and those with *CDKL5* mutations show similar sleep and breathing features. All our patients with *CDKL5* mutations showed disturbed sleep characterized by frequent awakenings, low sleep efficiency and decrease in REM sleep. During the night in our youngest patient with *CDKL5* mutations, obstructive sleep apneas (not clinically relevant) and some central apneas were present. Two of our patients had central apneas during the awake state. In classic Rett syndrome, respiratory evaluation is common practice. We suggest that assessment of sleep and breathing during the day and night is also warranted in patient with *CDKL5* mutations.
Literature


