Variant of Rett Syndrome and CDKL5 Gene: Clinical and Autonomic Description of 10 Cases

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Abstract

Rett syndrome (RTT) is a severe neurodevelopmental disorder affecting almost exclusively females. The Hanefeld variant, or early-onset seizure variant, has been associated with mutations in CDKL5 gene.

Aims In recent years more than 60 patients with mutations in the CDKL5 gene have been described in the literature, but the cardiorespiratory phenotype has not been reported. Our aim is to describe clinical and autonomic features of these girls.

Methods 10 girls with CDKL5 mutations and a diagnosis of Hanefeld variant have been evaluated on axiological and clinical aspects. In all subjects an evaluation of the autonomic system was performed using the Neuroscope.

Results Common features were gaze avoidance, repetitive head movements and hand stereotypies. The autonomic evaluation disclosed eight cases with the Forceful breather cardiorespiratory phenotype and two cases with the Apneustic breather phenotype.

Keywords
- CDKL5 gene
- Rett syndrome
- epilepsy
- autonomic nervous system

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Conclusions The clinical picture remains within the RTT spectrum but some symptoms are more pronounced in addition to the very early onset of seizures. The cardiorespiratory phenotype was dominated by Forceful breathers, while Feeble breathers were not found, differently from the general Rett population, suggesting a specific behavioral and cardiorespiratory phenotype of the RTT the Hanefeld variant.

Introduction

Rett syndrome (RTT) is a neuro-developmental disorder almost exclusively affecting females, characterized by a broad clinical spectrum. After the first identification of the gene in 1999[1] MeCP2 mutations are now identified in 90 to 95% of the cases with classic RTT. In this form, after a period of apparently normal development, patients show developmental delay and loss of acquired skills such as purposeful hand use and communication, a deceleration of head growth, and appearance of stereotypic hand movements, ataxia/dyspraxia and insufficient growth. Diagnostic criteria[10,19] and several RTT variants[9] have subsequently been described, including Zappella variant (preserved speech variant), characterized by the recovery of some degree of speech,[22] the congenital variant (recognized since birth and often caused by FOXG1 mutation),[3] and the "early onset seizure variant or Hanefeld variant.[4,11]

In 1984, Hanefeld described a girl with infantile spasms, whose onset was within the first years of life; this girl only subsequently disclosed symptoms of classic RTT with progressive drug-resistant epilepsy. In 2003, the Hanefeld variant was associated with the cyclin dependent kinase-like 5 gene (CDKL5), previously known as serine/threonine kinase 9 (STK9) and the first CDKL5 mutations were found in two girls with a West syndrome clinical picture (infantile spasms, hypsarrhythmia and severe mental retardation).[15]

CDKL5 is a putative serine/threonine kinase of unknown function, expressed in several cell lines and tissues in early developmental stages. CDKL5 is located in the cell nucleus suggesting a gene regulation function. It has been observed that MeCP2 and CDKL5 gene mutations lead to similar phenotypes, and thereby it was postulated that they both could be involved in the same signaling pathway. Since it has been demonstrated that MeCP2 is subjected to phosphorylation and that CDKL5 has a kinase-domain, it seems possible that MeCP2 is directly phosphorylated by CDKL5. Alternatively, CDKL5 might phosphorylate a third intermediate protein that could dephosphorylate MeCP2.

The clinical features identified in the patients reported in literature[15,17,21,24,25] are mostly of atypical RTT, myoclonic encephalopathy, or epilepsy and mental retardation or autism. Almost all patients show seizures during their lives with a median age of onset of 2 months. The seizure pattern is mainly characterized by infantile spasms, myoclonic jerks, generalized tonic-clonic seizures, tonic seizures and, to a lesser extent, absences and complex partial seizures. The majority of cases is resistant to drug therapy. The EEG pattern reported is also variable including a hypsarrythmic pattern, focal or multifocal activity, diffuse high-voltage sharp waves, and generalized theta rhythms.

The aim of the present study is to describe the clinical and autonomic features of 10 RTT girls with Hanefeld variant and CDKL5 gene mutation.

Materials/Subjects

We selected 10 patients with characteristics of atypical RTT (Hanefeld variant) and CDKL5 mutations: 8 of them selected from 119 cases referred to the Rett Centre at Versilia Hospital (Lido di Camaiore, Tuscany) from October 2006 to December 2009; the other two from a population of 115 RTT patients referred to the Swedish Rett Center (Sweden).

Hanefeld variant in the Italian sample was 8.4%, in the Swedish 5.3%.

All cases with CDKL5 are female, the mean age is 7.7 years (range 2 to 13).

Clinical and genetic features are reported in ►**Tables 1** and **2**.

Methods

The diagnosis of Hanefeld variant was based on established clinical criteria.[4] All subjects had been investigated by molecular analysis of MeCP2 or CDKL5 gene. In our sample of Hanefeld patients, all subjects had a CDKL5 mutation.

Our patients were evaluated clinically using the International Scoring System (ISS).[16] Their cardiorespiratory phenotype was investigated with the Neuroscope (Medifit Instruments Ltd, London, UK).[12,14]

Clinical Score

ISS consists of 21 items regarding the typical characteristics of RTT, divided in five subscales: Growth and Development, Muscolo-Skeletal appearance, Movement, Mental-Cortical and Brainstem-Autonomic. Each item score ranges from 2 to 0 as follows: 2, severe abnormality; 1, mild abnormality; 0, no abnormality.

Neuroscope

Autonomic and cardiorespiratory function indexes were recorded non-invasively and synchronously with EEG and video over a 1-hour period. The autonomic parameters included: cardiac vagal tone, heart rate, transcutanoeus blood gases, and respiratory patterns. Following analysis of the recording each patient was assigned to a specific cardiorespiratory phenotype according to her respiratory dysrhythmia.

Table 1 Clinical and Autonomic Features

Features	1	2	3	4	5	6	7	8	9	10
Age at evaluation (years)	5	13	6	10	4	2	12	4	10	11
OFD at birth	38 cm (gestational diabetes)	34 cm	34 cm	34.7 cm	35.8 cm	35.5 cm	34 cm	35 cm	36 cm	35 cm
Acquired microcephaly	No	Yes	Yes	No	No	No	No	No	No	No
Degree of psychomotor delay	Severe	Moderate	Moderate	Severe	Severe	Severe	Moderate	Moderate	Severe	Severe
Age of onset of epilepsy	1½ month	1 month	1½ month	3 months	3½ months	3 months	6 months	2 months	1 month	1 month
Type of epileptic crisis at the onset	Generalized tonic-clonic	Spasms	Clonic	Clonic	Clonic	Partial (clonic movements right sided)	Spasms	Partial (adversive seizures and right sided clonic movements)	Respiratory arrest	Eye-blinking muscle-twiches in all four extremities
Type of epileptic crisis in the following period	Spasms	Generalized tonic-clonic	Spasms	Spasms, tonic, versive	Partial	Spasms, Tonic	Atonic seizures	Complex partial seizures	Quick muscle twiches mainly in the arms	Tonic-clonic seizures, myoclonia and eye-blinking
EEG evolution	Diffuse aspecific abnormalities	Spikes > occip, slow background	Diffuse aspecific abnormalities	Diffuse epileptic abnormalities	Generalised spike and wave activity	Left centro-temporal abnormalities	Diffuse abnormalities and multifocal spikes	Normal	Generalized spike and wave activity	Normal
Epilepsy	Partially controlled	Partially controlled	Refractory	Refractory	Partially controlled	Seizure free	Partially controlled	Seizure free	Refractory	Refractory
Brain RMN abnormalities	↑ Lat ventricular and pericerebral space	Absent	Absent	Mild occipital horns ssymmetry	Absent	Mild lateral ventricular asymmetry	Increase of frontal sub-arachnoid spaces and sylvian Scissure	Mild lateral ventricular asymmetry	Normal, except for minimal liquified structure left, frontal part of Capsula interna.	Normal
Visual defect	No	No	Central visual defect, horizontal nystagmus	Squint, nystagmus	Squint	Squint, horizontal and torsional nystagmus	Myopia	Squint	Slight squinting	Hyperopia
Type of cardiorespiratory phenotype	Forceful	Forceful	Forceful	Forceful	Forceful	Forceful	Forceful	Apneustic	Apneustic	Forceful
Type of CDKL5 mutation	Missense (R178W)	Frameshift (R55fsX74)	Nonsense (E364X)	Del exons 7–8	Missense (N71D)	Del exons 1–10	Frameshift (D618EfsX620)	Frameshift (N314fsX349)	Missense (R175S)	Missense (I72N)
ISS	25	18	28	17	14	17	16	14	21	33

Thirteen abnormal, awake breathing rhythms have been identified in the RTT population and categorised into Feeble, Forceful, and Apneustic types of breathing. The RTT population can be subdivided into these three groups of breathing types. They constitute three unique cardiorespiratory phenotypes with different levels of blood gases, autonomic tone, physical features, clinical complications and idiosyncratic responses to drugs. Rational approaches to clinical management are different and unique for each phenotype.[13]

Results

The clinical and autonomic characteristics of our sample are summarized in ►**Table 1**.

All cases are female, the mean age is 7.7 years (range 2 to 13).

All subjects had an identified mutation in the CDKL5 gene (N71D, I72N, R175S, R178W, E364X, R55fsX74, N314fsX349, D618Efs620X, deletion of exons 7–8, deletion of exons 1–10).

The mutations observed can be divided into the following groups: missense (4 cases), frameshift (3 cases), nonsense (1 case), deletion (2 cases). At birth the head circumference (HC) of 9 patients was within the normal range, in 1 case at the 95th centile (gestational diabetes was diagnosed during pregnancy).

The head growth was normal in 8 cases while 2 cases presented acquired microcephaly. In the first year all patients showed a delay in developmental milestones: among them 8 patients presented generalized hypotonia, with antigravitary activity, whereas 2 had a normal muscular tone and they acquired antigravitary, crawling and locomotion, but they showed an ataxic gait. At the last evaluation, 6 patients had an ataxic but unaided gait, while the others were unable to walk (►**Table 2**). All patients had mental retardation, corresponding to 1 and 2 in ISS; speech was absent in all patients. Some kind of alterations of the visual system (nystagmus, squint, myopia, hyperopia) were found in 8 of 10 cases.

From the behavioral point of view, common features were mood disorders, gaze avoidance and hand stereotypies (hand-mouthing, hand-washing, hand-clapping, often observed in combination in the same subject); in addition, in 5 cases, repetitive head movements were noted. Self injury behavior was rarely present in our sample. All subjects had early onset epilepsy: with seizure onset in 3 cases in the first month, in 3 cases the second month, in 3 cases, the fourth month and in 1 case the sixth month. Mean age at seizure onset was ~2 months. Seizures at onset were predominantly generalized tonic-clonic, infantile spasms, clonic seizures (in two cases multifocal and in another case involving lower limbs synchronously). After the first year of age, the seizures evolved into infantile spasms, generalized tonic-clonic, partial seizures and, in one case, tonic-adversive. According to Bahi-Buisson[5] we subdivided our patients into three categories on the basis of the antiepileptic drug response: 4 patients were drug resistant, 4 had partially controlled seizures (frequency once in a month to once in a year) the other 2 were seizure free at least from one year. The interictal EEG pattern showed diffuse spike and wave activity, predominantly over the fronto-temporal/centro-temporal areas in 3 cases and on the occipital area in 1 case; 4 patients did not show significant epileptic activity on the EEG.

Cerebral MRI was normal in 5 cases or showed only non-specific abnormalities such as a mild increase of lateral ventricle size and periencephalic spaces around the fronto-temporal regions in 4 cases, and in another, mild asymmetry of the occipital horns (►**Table 1**).

The Sleep-Wake Rhythm (SWR) in RTT patients showed irregularity in the time of waking up and of falling asleep at night and also profound daytime sleep by comparison with normal children of the same age. Abnormalities in the SWR are considered to be due to abnormalities of the serotonin (5HT) neurons which develop in early infancy before 4 months and also in late infancy around eight months and suggest involvement of these neurons in these disorders.[20,23]

In the anamnestic datas sleep had been an important problem from an early age in all the patients. In the first 4 months of life they all had sleeping problems. There were severe derangements in the sleep-awake rhythm, they could be awake for several days and nights in periods and sleep day and night in other periods. They had also been woken up at night by seizures. Three patients had regular sleep-awake cycles (1, 4, 6, ►**Table 2**), one of them aged 2 years; the others, from 4 to 10 years, continued to present sleep problems even if milder than in the first months of life.

The cardiorespiratory phenotype was Forceful in 8 of 10 cases and Apneustic in 2 of 10.

No cases with feeble breathing were present in our sample. Cardiorespiratory dysrhythimia was present during 37.9% of the one hour recording on an average. The most frequent dysrhythimias were tachypnoea (11.7%), deep breathing (4%) and breath-holding (3.25%); less common were Valsalva maneuvers (0.97%) and apnoea (1.4%). The mean vagal tone was 5.2, range 2.2 to 7.6 in the linear vagal scale (LVS) (normal range: 6 to 19 LVS); the maximum vagal tone was 26.7, range 7 to 40.

The total score of the ISS varied from mild to severe (range 14 to 33), in contrast to the score, generally severe, found in most patient with classic RTT. There was a wide variability of the cognitive levels in the subjects: mental disability varied from severe to moderate with acquired abilities as reading and writing with alphabet tables.

Discussion

All patients in this study had the characteristics of atypical RTT and this suggests that the CDKL5 molecular analysis should be performed not only in all subjects with the clinical suspicion of the Hanefeld variant but also in MeCP2 negative cases. On the other hand, the first case described by Hanefeld[11] had a MeCP2 gene mutation (personal communication) which may occasionally result in this variant.

Furthermore, a Rett girl with atypical RTT and mutations in both genes (although in the end the MeCP2 was considered as very rare polymorphism) has also been described[7] suggesting the possible coexistence of the CDKL5 mutation and the MeCP2 mutation as well as a clinical overlap between the

Table 2 Behavioral and Motor Phenotype

Features	1	2	3	4	5	6	7	8	9	10
Gaze avoidance	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Lactatio capitis or head movements	Yes	Repetitive head rotation	No	No	Yes	No	Rapid head flexion	Occasional head hyperextension	No	No
Repetitive behavior	Truncal rocking motions	No	Truncal rocking motions	No	No	No	Yes	No	Yes	Yes
Mood disorder	No	Yes	Yes	No	No	Yes	No	Yes	No, easily agitated	No, easily agitated
Self-injuries behavior	No	Yes	No	No	No	No	No	Yes	No	No
Type of hand stereotypies	Hand-mouthing, autistic hand-flapping	Hand-washing, hand-mouthing	Hand-washing hand/nose-mouthing scratching	Hand-washing, hand-mouthing	Hand-mouthing	Hand-clapping	Hand-washing, Hand-clapping	Autistic hand-flapping	Hand-mouthing	Handclapping
Hypotonia	Yes	no	Yes	Yes	No	Yes	Yes	Yes	No	No
Crawl	No	No	No	No	No	No	Yes	Yes	No	No
Locomotion	No	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes
Abnormality sleep-wake (0–4 months)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Abnormaliy sleep-wake (> 4 months)	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes

two conditions. In cases of negativity in both genes, a further investigation of FOXG1 gene could be useful, because epilepsy may begin even earlier in the congenital variant.

The phenotype of the RTT of our sample was dominated by early onset epilepsy which is rare in girls with classic RTT due to MeCP2 mutations. This is the main distinctive feature of the Hanefeld variant, reported in the literature. Our patients present many clinical characteristics typical of RTT such as delayed mental and motor development, loss of hand function, hand stereotypes and absence of speech; normal HC at birth and subsequent deceleration of HC growth was found in only a minority of cases.

There was a great variability in the clinical picture: six patients had mild ataxia, while the other four had severe motor impairment with an inability to walk. The hand stereotypes varied considerably in type and severity. In 5/10, the presence of repetitive head movements was noted: among them, 3 cases presented some kind of visual defect (squinting in 2 cases and myopia in 1 case).

We found 4 types of seizures at presentation: generalized tonic-clonic (1 case), clonic seizures (5 cases), partial tonic seizures (1 case), "respiratory arrest" seizure (1 case) and infantile spasms (2 cases). At the time of the clinical assessment, there was a great variability in the type of seizures with generalized tonic-clonic, infantile spasms, partial seizures and atonic seizures in contrast to that suggested by other authors who described patients with CDKL5 mutations as having myoclonic encephalopathy syndromes.[6]

A characteristic electroencephalographic pattern was not seen. There were diffuse unspecific abnormalities only in two patients; six patients had epileptic discharges (five bilateral and one multifocal) and two were normal. Archer et al. reported that temporal or fronto-temporal activity was the most common and could suggest a focal pathology, in agreement with experimental studies showing that the highest CDKL5 expression is reached in the first postnatal days in the neocortex, the piriform cortex, and in the medial temporal lobe structures.[2]

Most CDKL5 patients (9 of 10) were in politherapy, unlike classic RTT where seizures were usually treated in monotherapy with good results.

Differently from other reports[18] the patients in our study showed symptoms of dysautonomia such as cold hands or feet, constipation, diurnal dysrythmia and breathing dysrythmia such as hyperventilation, breath holding and Valsalva maneuvers.

No cases with feeble breathing were found; the most frequent type of respiratory dysrythmia was Forceful (8 of 10) while two cases were Apneustic breathers.

CDKL5 molecular analysis showed several kinds of mutations. Three cases with "missense" mutations (all localized in the catalytic domain of the CDKL5 protein) did not show a mild phenotype in contrast to previous reports.[8,24] In these cases the range of ISS was broader (14 to 33) if compared with other classes of mutations; in addition, some autistic traits like gaze avoidance and impairment in social interaction were also very evident. The case with the "non sense" mutation had a severe ISS, comparable with the higher range of the missense mutations. The "frameshift" mutations are in general known to lead to a loss of function of the CDKL5 protein; lack of functional CDKL5 protein is reported to cause severe Infantile Spasm syndrome X linked (ISSX).[24]

In our sample the ISS in patients with frameshift mutations is in the range of mild-moderate severity (14 to 17); among them we want to report one case with neuromotor delay, but ability to walk at 6 years and good seizure control (started at 1 month). The same range of severity (of the moderate degree) characterized all the cases with the deletion type of mutation.

These data are not sufficient to draw firm conclusions but could represent a contribution to definite genotype-phenotype correlations.

Conclusions

As MeCP2 gene mutations are not synonymous with Rett syndrome so CDKL5 gene mutations are not synonymous with the early-onset seizures variant. The term "Hanefeld variant" should be reserved for patients with clinical features of Rett syndrome, with epileptic encephalopathy before 6 months of age, regardless the presence of genetic abnormalities (mutations in MeCP2 or CDKL5 or both) or even in their absence.

In patients with CDKL5 mutations, seizures are not always intractable and cognitive levels are highly variable.

There is a possible relationship with the Forceful and Apneustic cardiorespiratory phenotype.

Clinically, gaze avoidance and repetitive head movements were common in addition to the typical RTT signs.

We believe this study will contribute to delineate a specific behavioral and cardiorespiratory phenotype of the RTT syndrome, the Hanefeld variant.

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