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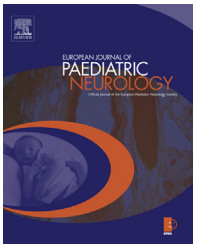

Original article

Is it possible to diagnose Rett syndrome before classical symptoms become obvious? Review of 24 Danish cases born between 2003 and 2012

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ABSTRACT

Background/Purpose: Rett syndrome (RTT) is a neurodevelopmental disorder that affects mainly females; it results in multiple disabilities and carries a risk of medical comorbidities. Early diagnosis is important to help establish the best treatment opportunities and preventive care in order to slow down the progression of symptoms. We wanted to test our hypothesis that it is possible to diagnose RTT before the classical symptoms become obvious.
Methods: We analysed development and symptoms before and at the time of the RTT diagnosis, as well as the symptoms that triggered *MECP2* mutation analysis, in a cohort of girls with RTT born in Denmark between 2003 and 2012.
Results: Twenty-four girls were included, and 87.5% of these girls were diagnosed when the classical RTT symptoms were recognized. However, parents were concerned about their daughters between 3 and 58 months prior to the RTT diagnosis, and they felt that the professionals did not share their concern in the beginning. When reviewing medical files and questionnaires, we noted that the majority of girls did have combinations of concerning symptoms such as developmental delay and a collection of subtle signs such as autistic traits, placidity, floppiness with suspicion of muscular or mitochondrial diseases, hair pulling, teeth grinding, development of incontinence and problems with initiating movements.
Conclusion: We conclude that many individuals with *MECP2* mutation exhibit characteristics that should raise suspicion for RTT, prior to evolution of the core clinical criteria. As RTT is a rare disease, it is of importance to constantly educate clinicians for heightened awareness of RTT.

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1. Introduction

Clinically diagnosing a rare disease such as Rett syndrome (RTT) requires knowledge of the specific symptoms and course of the condition. Paediatricians and clinical geneticists who deal with intellectual disability (ID) are aware of RTT in girls when the classical symptoms have appeared.[1]

However, the diagnosis is often delayed in relation to onset of symptoms and might be missed especially in girls with an atypical course of the disease.[1,2] Previous studies of girls with moderate to severe ID without prior suspicion of RTT have shown an average of 1.5% *MECP2* mutations.[3–6] Regarding medical genetic evaluation of females with global developmental delay, the guidelines of the American Academy of Pediatrics recommend considering RTT in females if no diagnosis is established after the initial evaluation (microarray, Fragile X and metabolic testing).[5]

RTT is a neurodevelopmental disorder that affects mainly females; it results in multiple disabilities and carries a risk of medical comorbidities. It affects approximately 1:10,000 live female births, the majority of cases having a mutation in the methyl-CpG-binding protein 2 (*MECP2*).[2,7,8] During the first 6–18 months of life, development is apparently normal, followed by stagnation and loss of many acquired skills. Diagnosis is based on clinical criteria. Typical RTT is characterized by regression of purposeful hand use and spoken language, development of abnormal or absence of gait, and hand stereotypies. Atypical RTT is characterized by at least two of these four criteria and at least 5 of 11 supportive criteria, according to diagnostic criteria, 2010.[8]

There are many reasons why it is important to diagnose developmental diseases such as RTT as early as possible. This will, for instance, provide the patients and families with more specific counselling concerning the course of the disease, symptom surveillance, prevention and treatment, as well as specific genetic counselling and family support.[5] The importance of early diagnosis will become even more pronounced in the future when we have learned to treat RTT in a more curative way.

Several studies published in the years 1990–2014 show that early development in girls with RTT might be abnormal before the onset of classical symptoms. This relates to milestone attainment and behaviour,[9–16] communication,[17–19] movements and muscle tone.[20–22] However, we still lack knowledge of the combination of very early signs of RTT. One study has shown that some girls with RTT are diagnosed with autism before the diagnosis of RTT.[23] This implies that symptoms from the autism spectrum may contribute to the picture of symptoms of RTT together with less specific symptoms and developmental delay.

We hypothesize that it is possible to diagnose RTT clinically before the classical symptoms, including regression, become obvious. We therefore conducted a study to analyse the girls' development and symptoms before and at the time of diagnosis, as well as the symptoms that triggered *MECP2* mutation analysis, in order to better describe combinations of disturbing symptoms that should lead health care professionals to consider RTT as early as possible.

2. Material and methods

2.1. Participants and clinical evaluation

Girls with RTT known by the national Centre for Rett Syndrome (CRS), born in Denmark since 2003 and identified with a *MECP2* mutation were included if clinical data were sufficient for analysis. Mutation analysis of *MECP2* in Denmark is performed only at the Department of Clinical Genetics, Rigshospitalet, which allowed us to gather knowledge of all *MECP2* mutations performed in Denmark. Even though the RTT diagnosis is a clinical diagnosis, the genetic analysis is part of the diagnostic evaluation in Denmark as mutation analysis is easy and affordable. Individuals diagnosed with RTT are referred to CRS for clinical investigation and genetic and clinical counselling, as well as data collection. CRS was established in 2007 and offers lifelong multidisciplinary follow-up and counselling.

The girls were classified as having typical or atypical RTT according to the diagnostic criteria for RTT, 2010.[8]

Even though RTT is a clinical diagnosis, the majority of the girls were diagnosed with RTT at local paediatric departments after the *MECP2* analysis had shown a mutation. We therefore defined time at diagnosis as the date of the mutation report. We are aware that this overestimates the real age at diagnosis.

2.2. Data collection

We did a search (September 2014) in the Danish database for RTT at CRS of age (born 2003–2014), mutations, early development and symptoms, as well as parents' concern before the diagnosis. We reviewed medical files from local paediatric departments and CRS, as well as the indications for *MECP2* mutation analysis and questionnaires completed by the parents.

The clinical evaluations included videos of the girls and the Severity Score (Kerr, 2001),[24] Clinical Severity Score (CSS) (Neul/Percy)[25] and gross motor function classification system[26,27] in order to classify the girls according to severity at the time of the diagnosis. Gross motor function classification (GMFCS) is a five-level classification system looking at self-initiated movements and was developed for children with cerebral palsy. Level five includes the most severely affected children. We modified the GMFCS to allow initiation by a therapist, as it is a huge problem for persons with RTT to initiate movements. The Severity Score (Kerr) describes 20 clinical RTT features, each ranging from 0 to 2 with 2 being the most severe. The CSS (Neul/Percy) was also developed for RTT; 13 disease-related items are scored from 0 to 5 with 5 being the most severe.

3. Results

3.1. Participants, regression and diagnostic evaluation, Table 1

The RTT database included 24 girls born between 2003 and 2012 and with a *MECP2* mutation. Sufficient information was

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Table 1 – Participants, regression and diagnostic evaluation, arranged according to age at *MECP2* result.

ID	age at *MECP2* (months)	year for MECP2	parents' or caregivers' concern "that something was wrong" before RTT diagnosis (months)	age at regression	diagnosis in relation to regression	*MECP2* mutation	typical or atypical (criteria 2010)
1	15	2006	7	10	under	c.378-?_*?del	ty
2	16	2010	8	12	under	c.378-3C>G	ty
3	17	2011	8	12	under	p.R168X	ty
4	18	2012	6	15	under	p.T158M	ty
5	19	2009	9	18	under	p.T158M	ty
6	19	2004	12	23	before	Q128X	ty
7	20	2008	?	?	?	p.R168X	ty
8	21	2009	14	15	under	p.T158M	ty
9	24	2005	12	12	after	p.R168X	ty
10	24	2014	12	20	under	p.R168X	ty
11	26	2013	14	16	under	c.1155del12	ty
12	27	2005	11	21	under	p.T158M	ty
13	28	2010	14	24	under	p.R270X	ty
14	29	2010	3	30	before	p.T158M	ty
15	30	2011	18	20	under	c.27-?_*?del	ty
16	30	2007	18	18	under	p.R168X	ty
17	30	2014	23	12	after	p.P152R	ty
18	32	2012	24	9	after	c.806delG	ty
19	36	2010	21	15	under	c.27-?_*?del	ty
20	42	2013	27	15	after	p.L100V	ty
21	43	2014	28	21	after	p.T158M	ty
22	54	2009	?	30	after	c.1164del44	ty
23	62	2012	50	30	under	p.F157L	aty
24	64	2013	58	42	after	c.1163del35	aty

Mutation group

Early truncated for NLS
Late truncated for NLS
C-terminal deletions
Missense

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Table 2 – Symptoms observed before RTT diagnosis.

ID	Early specific RTT symptoms			Unspecific but recurrent disturbing symptoms								First diagnostic approaches	
	Abnormal hand movements or hand skills	Signs of developmental arrest	Less or poor social interaction	Developmental delay	Floppy	Hypermobility	Placid behaviour the first 1/2 year	Gastrointestinal reflux	Squint	Teeth grinding	Other symptoms	Referred to psychiatric department before RTT diagnosis	Investigation for muscular or mitochondrial disease
1	x	x		x	x	x	x						
2			x	x	x				x		Minor movements of legs		
3				x	x	x	x						
4		x	x				x						
5		x		x	x	x	x		x	x	Hair pulling		x
6			x	x	x		x			x			
7				x	x[a]		x						x
8	x				x[a]		x		x	x	Night screeming		x
9	x		x	x	x	x	x	x			Myopic astigmatism, recurrent infections		
10	x			x	x	x	x				Hair pulling		x
11				x	x[b]	x	x				Shivering on awakening	x	
12				x	x	x	x	x			Progressive microcephaly, epilepsy		
13		x	x		x[b]	x					“Freaks out”, difficult to comfort	x[c]	
14				x		x	x		x		Hypermetropia		
15	x			x		(x)	x				Hair pulling, uncritically puts thing i mouth, less social interest		
16	x	x	x	x	x	x	x	x		x	Microcephaly		
17			x	x	x		x		x				x
18			x	x	x	x	x		x		Microcephaly, ataxia, failure to thrive		x

19	x	x			x[b]					x			
20	x	x	x	x		x	x				Sleeping disorder, hair pulling	x	
21			x	x			x				Screeming attacks	x	
22	x	x			x	x	x				Toe walking, seizures	x[c]	
23		x		x				x			Development of incontinence, epilepsy	x	
24		x			x	x	x	x	(x)		Development of incontinence, minimal interest in toys, recurrent infections	x	x

a Severe floppiness/hypotonia.
b Mild floppiness/hypotonia.
c Diagnosis of autism.

available for all 24, who therefore were included in the present study. The girls were molecularly diagnosed in 2004–2014; the incidence was 1.25/year until 2007 and 2.7/year in 2008–2014. All girls but one had a clinical and molecular RTT diagnosis before the first evaluation at CRS, which ranged from 13 days to 46 months after the result of the *MECP2* mutation analysis. Ten of the girls were evaluated within two months (13–57 days). One girl was seen before the mutation analysis result, and the clinical diagnosis was made at CRS. The evaluations were performed by one of three physiotherapists, all experienced in RTT, and all girls have been followed by paediatricians (Jytte Bieber Nielsen, authors AMB and GR).

The age at diagnosis of all 24 girls ranged from 15 to 64 months (mo); median 27.5 mo; mean 30.25 mo. The seven girls (Tables 1 and 2) who were referred to a psychiatric evaluation were diagnosed at age 26–64 mo; median 43 mo; mean 45.6 mo. Parents' or caregivers' concerns that “something was wrong” appeared 3–58 mo before RTT diagnosis (N = 22); median 14 mo; mean 18.05 mo. Age at regression ranged from 9 to 42 mo (N = 23); median 18 mo; mean 19.1 mo. Two girls had *MECP2* testing before regression began, 14 girls during regression and 7 girls after the condition had stabilized. In one case it was not possible to define the period where she lost milestones. Twenty-two girls had typical and two girls had atypical RTT.

MECP2 mutations are shown in Table 1.

3.2. *Symptoms before the RTT diagnosis, Table 2*

Nineteen girls (79%) had symptoms often seen in RTT such as abnormal hand movements or hand skills, signs of developmental arrest and/or less or poor social interaction before the diagnosis of RTT was suspected. Furthermore, all but one girl were noticed to have some degree of developmental delay or developmental arrest. In 20 cases (83.3%) the parents described their child as being placid and easy to comfort in the first months of life. Nine girls (37.5%) had abnormal hand movements, hand stereotypies or a tendency to put their hands in their mouth in the time before RTT was suspected. One of these girls and three more had a tendency toward hair pulling, five girls were teeth grinding and two girls developed incontinence. There was a suspicion of muscular or mitochondrial disease in seven (29%) girls, and 11 more girls were described as being floppy (decreased muscle tone) to different degrees.

Seven (29%) of the girls were referred to psychiatric evaluation. Two of these girls were diagnosed with autism, and RTT was suggested in two other girls, leading to a *MECP2* investigation.

See Table 2 for further details. Symptoms occurring fewer than five times are mentioned under other symptoms.

3.3. *Symptoms leading to MECP2 mutation analysis, Table 3*

Mutation analysis was done in 21 cases (87.5%) when specific RTT symptoms such as hand stereotypies (N = 19, 79%), regression (N = 13, 54%) or abnormal breathing (N = 2, 8.3%) were recognized.

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Table 3 – Symptoms leading to MECP2 mutation analysis.

ID	Specific RTT symptoms			Unspecific but recurrent disturbing symptoms						Final diagnosis	
	Handstereotypies	Regression	Abnormal breathing	Loss of social interest/ changed behaviour	Autistic traits	Developmental delay	Microcephaly or decelerating head circumference recognized	Floppiness or ataxia	Epilepsy	*MECP2* because of specific RTT symptomsMECP2 because of specific RTT symptoms	Who though of RTT first
1	x		x		x	x		x		x	Neuropaediatric Department
2	x	x				x				x	Neuropaediatric Department
3	x	x		x		x			x	x	Neuropaediatric Department
4	x	x				x		x		x	Neuropaediatric Department
5	x			x		x			x	x	Neuropaediatric Department
6	x			x	x	x		x		x	Opthalmologist
7	x					x	x			x	Neuropaediatric Department
8	x	x		x		x				x	Neuropaediatric Department
9		x		x		x	x	x		x	Neuropaediatric Department
10	x				x	x			x	x	Parents
11	x	x		x	x	x				x	Psychiatric Department
12	x					x	x	x	x	x	Neuropaediatric Department
13	x	x		x	x	x				x	Neuropaediatric Department
14						x					Neuropaediatric Department
15		x				x				x	Neuropaediatric Department
16	x	x	x			x				x	Neuropaediatric Department
17	x					x		x		x	Parents
18	x					x				x	Neuropaediatric Department
19	x	x						x		x	Parents
20	x	x		x		x				x	Psychiatric Department
21	x	x		x		x				x	Parents
22				x	x	x					Neuropaediatric Department
23					x	x			x		Neuropaediatric Department
24	(x)	x		x	x					x	Psychologist

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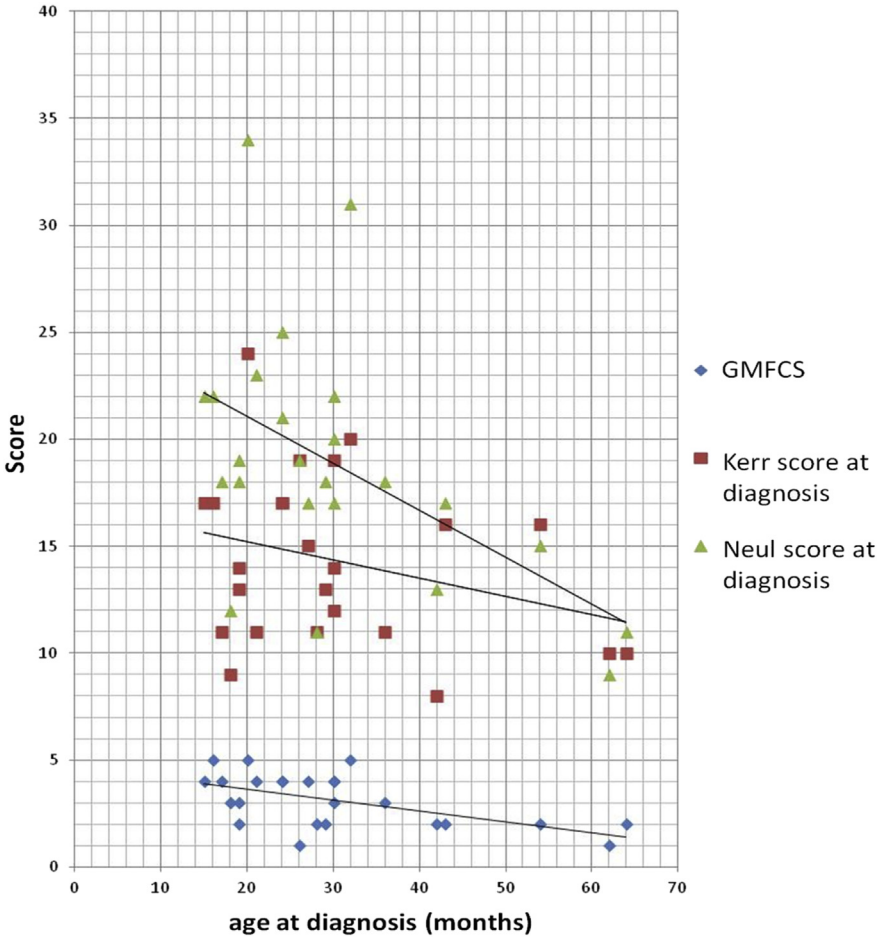

Fig. 1 – The linear diagram shows the severity scores from the three scoring systems correlated to age at diagnosis. Range of GMFCS is 1–5; Kerr score 8–24; Neul 9–34.

Other less specific symptoms that led to diagnosis were developmental delay (N = 22, 91.7%), loss of social interest/changed behaviour (N = 11, 45.8%) and specific autistic traits (N = 8, 33.3%). Five girls (20.8%) had epilepsy at the time of the diagnosis.

The table also shows that mainly neuropaediatricians made the diagnosis, but in four cases the parents found the diagnosis on their own; other specialists in psychiatry and ophthalmology also recognized the phenotype, as well as one psychologist.

See Table 3 for further information.

3.4. *Clinical severity at diagnosis, Fig. 1*

The linear diagram in Fig. 1 shows the severity scores according to three scoring systems: the Severity Score (Kerr), the CSS (Neul/Percy) and the modified GMFCS score assessed at the time of diagnosis.

There is a tendency toward increased severity score with a younger age at time of *MECP2* mutation screening, with the largest diversity in the CSS due to a larger total score possible. With a selected cut-off equivalent to the median age of diagnosis, 27.5 mo, the group of RTT girls diagnosed before had a median CSS of 20, a median Kerr score of 16 and a median GMFCS score of 4. The group of girls diagnosed after the median age of diagnosis had a median CSS score of 17, a median Kerr score of 12.5 and a median GMFCS score of 2.

Nine girls in all were able to sit and to walk independently or with minimal help, but the four youngest of these had problems initiating movements to the sitting and/or standing position.

4. Discussion

We wanted to see retrospectively whether it was possible to diagnose RTT before the classical symptoms became evident. We found that the girls in our study cohort had distressing symptoms several months before they were diagnosed with RTT. Many of these symptoms (Table 2) were even early specific RTT symptoms included in the clinical criteria, and except for one girl, all had early symptoms that would suggest a neuropaediatric evaluation. The symptoms caused concern among the parents. The issues of concern were mainly developmental delay, but inactivity, lack of interest and "easy to satisfy" also were mentioned. Many of the parents expressed the feeling that the professionals did not share their concern in the beginning. These observations are in line with previous studies. Tarquinio et al. describe it as the "wait-and-see" approach where the children are at risk for late

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diagnosis.[28] In an Australian study Fehr et al. found parental concerns within the first 10 months of birth and described that nearly three quarters of the parents felt the behaviour/development had never been "normal".[14]

The early unspecific but recurrent and concerning symptoms may occur among children with other syndromes and among normally developing children. Placid behaviour can be interpreted as an "easy child", squint and reflux are quite common among all children younger than 1 year of age and some children are not as socially active as others. The more disturbing symptoms of developmental delay, floppiness and hypermobility are also very nonspecific.

Several symptoms are described in the medical files without being related to RTT: hypotonia/floppiness, vomiting/gastro-oesophageal reflux, squint, abnormal hand movements, hair pulling, night screaming, teeth grinding, toe walking and development of incontinence. We would like to emphasize that about a third of the girls had teeth grinding and/or hair pulling, symptoms we regard as RTT characteristics, before RTT was considered. In addition, we recommend looking at the development of incontinence as a sign of regression. As RTT is one of the most common neurodevelopmental disorders in girls,[6] a diagnosis of RTT should also be considered and monitored closely when more disturbing symptoms of developmental delay occur, and a *MECP2* mutation analysis should be conducted as a part of an early diagnostic approach.[5] The Danish girls were diagnosed mainly after a neuropaediatric evaluation. A recent American study shows the same tendency that RTT diagnosis is typically made by subspecialists.[28]

By analysing our referrals for *MECP2* mutation analysis and medical files, we found that mutation analysis was requested owing mainly to RTT symptoms such as hand stereotypies and changed behaviour. Other symptoms were mentioned, such as developmental delay, decreasing head growth, ataxia and hypotonia.

However, in 11 of the 24 cases regression was not registered as an indication. Three of these girls were post-regression, five were in regression, two girls were preregression and in one girl the regression period was undefined. We think that the regression period might be overlooked as it is not always well defined or easy to recognize in the moment. One explanation could be that some girls experience slow regression as Lee et al. saw in five of 13 girls.[15]

The majority of the 24 girls were diagnosed during the regression period or after. It is not uncommon for girls to be diagnosed after the disease has stabilized, as also shown in the study by Fehr et al., where the majority of girls (222/250) were diagnosed after the regression.[14]

The early course of RTT in the four girls, diagnosed at the ages of 29, 54, 62 and 64 mo respectively, was characterized by late regression after the age of 30 months. This observation is in line with the results from the Australian study by Fehr et al., in which they showed that atypical presentations are associated with a delay in diagnosis.[1] As illustrated in Fig. 1, our results also show a trend of earlier diagnosis of girls with more severe courses as compared to girls with lower severity scores. We have not correlated this to mutation type because of our small cohort. However, for comparison, Cuddapah et al. found a correlation between severity of mutation and age at diagnosis when evaluating 1052 participants.[29]

Nearly a third of the Danish girls were referred for psychiatric evaluation because of autistic or changed behaviour without suspicion of RTT. Should girls with autism then be tested for *MECP2* mutations? Zappella et al. showed in 2003 that *MECP2* mutations can be found in girls with autism and a history of atypical RTT, but they did not find mutations in girls with autism stable over the years.[30] Young et al. evaluated 313 cases with RTT and found significantly milder RTT symptoms in cases with an initial diagnosis of autism.[23] The girls in our cohort who were referred to psychiatric evaluation were diagnosed later than the other girls. As we also found that the late-diagnosed girls had some of the lowest severity scores, we therefore agree with Young et al. concerning the recommendation that females who are initially considered to have autism be carefully monitored for the development of RTT symptoms.[23]

Our data show that the diagnostic rate of RTT was approximately 1:20,000 live Danish female births in the years before CRS was established. We have an every-year diagnostic rate of RTT of approximately 1:10,000 live Danish female births from the years 2009–2014. This could indicate that paediatricians and clinical geneticists who deal with ID have become more aware of RTT over recent years. The latest incidence figure corresponds well to the generally accepted incidence rate of approximately 1:10,000 live female births.[8]

Our study adds knowledge to the understanding of early development in girls with RTT as we have looked at parents' and caregivers' concerns prior to the diagnosis of RTT and reviewed medical files, including the indications for *MECP2* mutation analysis referrals. It is also a strength of the study that the girls were clinically evaluated by professionals experienced in RTT early after the diagnosis. Videos from the evaluations reduced the professionals' recall bias. Furthermore, the parents were told to fill out questionnaires regarding early development and concerns prior to the clinical evaluation. It is a limitation that the methodology excludes the individuals with RTT who do not have an identifiable *MECP2* mutation. Furthermore, only 24 girls are included in the study. This low number is partly because we included girls only up to 11 years old in order to reduce the risk of recall bias and partly because the Danish population is 5.6 million people.

Children with *MECP2* mutation may not develop clinical criteria for RTT,[28] either because they are too severely affected and never achieving milestones that could later be lost, or because their phenotype is so mild that they do not lose skills. This limits the prognostic usefulness of identifying a *MECP2* mutation prior to clinical diagnosis. However, all anticipatory guidance associated with having a *MECP2* mutation still apply to such an early diagnosis. Hopefully, future studies will collect further data on early signs and symptoms in individuals with *MECP2* mutations with or without the core clinical criteria to contribute to revision of the clinical criteria.

5. Conclusion

Many individuals with *MECP2* mutation exhibit characteristics that should raise suspicion for RTT, prior to evolution of the core clinical criteria. The majority of girls have a collection of subtle signs, and in this respect, we would also like to draw

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attention to symptoms such as autistic traits, placidity, floppiness with suspicion of muscular or mitochondrial diseases, hair pulling and/or teeth grinding, as well as development of incontinence and, last but not least, problems with initiating movements. A *MECP2* mutation analysis should be considered early in the diagnostic evaluation if other genetic tests (microarrays, karyotype, Fragile X) are negative in these girls. Early diagnosis of RTT is important to help establish the best treatment opportunities and preventive care in order to slow down the progression of symptoms classical of RTT.

As RTT is a rare disease, it is of great importance to constantly educate paediatricians and clinical geneticists for heightened awareness of RTT.

Conflict of interest

There are no conflicts of interest.

Acknowledgement

We are grateful to our colleagues at the Danish Paediatric Departments for referring their patients with RTT to the Rett Centre and to the families who allowed us to evaluate their children. We also want to thank former and current employees at Centre for Rett Syndrome for their clinical evaluations and collection of data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejpn.2015.07.004.

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