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MECP2 Mutations in People without Rett Syndrome

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Abstract

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Mutations in *Methyl-CpG-Binding protein 2* (*MECP2*) are commonly associated with and the neurodevelopmental disorder Rett syndrome (RTT). However, some people with RTT do not have mutations in *MECP2*, and interestingly there have been people identified with *MECP2* mutations that do not have the clinical features of RTT. In this report we present four people with neurodevelopmental abnormalities and clear RTT-disease causing *MECP2* mutation but lacking the characteristic clinical features of RTT. One patient's symptoms suggest an extension of the known spectrum of *MECP2* associated phenotypes to include Global Developmental Delay (GDD) with Obsessive Compulsive Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD). These results furthermore reemphasize that RTT should remain a clinical diagnosis, based on the recent refurbished consensus criteria.

Keywords

Rett syndrome; autism; neurodevelopmental disorders; *MECP2*; epigenetics; neurogenetics

Rett syndrome (RTT, OMIM #312750) is a severe neurodevelopmental disorder that predominantly affects girls and, after Down syndrome, is believed to be the second leading known cause of mental retardation in women (Neul and Zoghbi 2004). The diagnosis of RTT is based on clinical features (Neul et al. 2010). People with RTT are born after an uneventful pregnancy and have apparently normal initial psychomotor development. However, development stagnates between 6-18 months. A marked regression, one of the defining features of RTT, occurs and is characterized by loss of purposeful hand use and spoken language. Ambulation is impaired, and characteristic repetitive hand stereotypies ensue. During regression many RTT patients exhibit social withdrawal which, occurring after a period of normal development, often leads to an initial diagnosis of autism (Young et al. 2008; Olsson and Rett 1987; Percy et al. 1988; Trevathan and Naidu 1988). However, once the characteristic hand stereotypies and other stigmata of RTT manifest, the diagnosis of RTT is often clear. Patients that do not fulfill all of the criteria of typical (or classic) RTT, but still have a clear regression of either hand skills or spoken language, may fall in the atypical RTT category, based on several additional features such as breathing abnormalities, sleep disturbances, cold and often small feet, and bruxism. All types of RTT often show intense eye-based communication (“eye-pointing”).

In the vast majority of cases (Neul et al. 2008), typical RTT is caused by mutations in *Methyl-CpG-binding protein 2*, *MECP2* (Amir et al. 1999), which encodes MeCP2, a protein that likely acts as a regulator of transcription (Chahrour and Zoghbi 2007). Although over two hundred different genetic changes in *MECP2* have been associated with RTT (RettBASE: http://mecp2.chw.edu.au), almost 70% of all sporadic mutations found in typical RTT arise from C-T transitions at only eight specific sites: four missense mutations at p.R106W, p.R133C, p.T158M, p.R306C, and four nonsense mutations at p.R168X, p.R255X, p.R270X, and p.R294X (Neul et al. 2008). In addition to these specific hot-spot point mutations, two other common mutational groups can be formed that result in similar molecular changes to the coding sequence and are expected to disrupt MeCP2 function in similar ways. The first are a group of small insertions or deletions in the C-terminal domain which all result in frameshift mutations and early truncations. People with these “carboxy-terminal truncations” have similar clinical features (Neul et al. 2008). The other mutational grouping is large DNA deletions that remove the majority of the coding sequencing of *MECP2* and are expected to completely eliminate all MeCP2 protein (Neul et al. 2008).

Despite this strong correlation between mutations in *MECP2* and RTT, mutations in *MECP2* are neither necessary nor sufficient for the diagnosis of RTT. There exists a wide variability of presentation in RTT (Bebbington et al. 2008), and approximately 5% of people with

typical RTT do not have any identified mutation in *MECP2*. Furthermore, there have been reports of other neurodevelopmental disorders associated with mutations in *MECP2*, including nonsyndromic autism and Angelman-like syndrome (Carney et al. 2003; Harvey et al. 2007; Watson et al. 2001). In this report, we further broaden the clinical spectrum of patients with *MECP2* mutations. We describe three novel presentations in people who clearly do not satisfy the criteria for diagnosis of typical or atypical RTT but have common RTT-causing mutations in *MECP2*, and one presentation that did not meet the criteria for RTT until she displayed very late and mild motor regression at 10 years of age. These cases emphasize that a mutation in *MECP2* alone is not sufficient for diagnosis of RTT and *MECP2* mutations should be considered in people with neurodevelopmental problems without the defining clinical features of RTT.

Methods

This study was approved by the Baylor Internal Review Board. We reviewed medical records of people who had presented to the Blue Bird Circle Rett Clinic at Texas Children's Hospital to identify individuals who carry known pathogenic *MECP2* mutations but lack defining features of RTT. The chart review focused on clinical course and signs of regression, as well as MRI and EEG results.

MECP2 mutational testing was performed by clinical laboratories. X chromosome inactivation (XCI) studies in peripheral blood lymphocytes were performed using standard methods established at the Baylor Molecular Genetics Lab(Allen et al. 1992).

Structured psychological assessment of these patients for autism included, an Autism Diagnostic Interview (ADI-R) as well as an Autism Diagnostic Observation Schedule (ADOS), performed by skilled child psychiatrists at Texas Children's Hospital, the Raleigh Developmental Evaluation Center (DEC), and the Calgary Child Development Centre. Patients were also all evaluated and followed by at least one pediatric neurologist with expertise in Rett syndrome and related disorders.

Patient SB had Bayley Infant Scales of Development and the Psychoeducational Profile-Revisited (PEP-R) performed in addition to ADOS and ADI testing. AK underwent Conners, Adaptive Behavior Assessment System (ABAS-II), Behavior Assessment System (BASC-II), and Differential Ability Scales (DAS-II) testing.

Results

Patient KR

Developmental History—KR was the third child of healthy parents and the product of an uncomplicated term pregnancy and birth. She started walking at 15 months of age but could not climb stairs until the age of seven. It was noted at that time that she had a normal base and station without evidence of ataxia. She continued to have difficulty with stair descent until 13 years of age. By age 13 she was able to walk and run by herself, as well as walk normally up- and down-stairs.

KR first started to grasp and hold objects at eight months. By age four, she was able to use a spoon to bring food to her mouth. She was able to hold a pencil at eight years of age, and by age 12 she was able to transfer objects between hands. By age 13 she could feed herself using knife and fork; however, she did not develop a mature pincer grasp until 16 years of age. KR started saying her first words at five years of age, had several words by age eight, and developed a vocabulary of 25 words by age 12. At that time she also started to speak in two- to three-word sentences and was noted to be functioning at the level of a five year old.

By age 16 she had acquired a vocabulary of about 100 words, knew colors and body parts. She had a severe articulation disorder coupled with unusual verbalizations.

KR's social communication was unusual; at 12 years of age she still patted her mother to get her attention and had almost no eye contact. This however improved as she was noted to have frequent eye contact with mother and examiner at an office visit at age 14. She never displayed the intense eye-based communication that is common in RTT. KR never developed any of the hand-stereotypies characteristic of Rett syndrome, though she occasionally flapped her hands at the sides and had finger rubbing when excited. Despite the delayed acquisition of psychomotor skills, KR **never displayed any regression**. KR frequently had cold extremities without them turning blue. She also had an increased pain tolerance, sometimes scratching herself to the point of bleeding. At 12 years of age she was noted to have weekly seizures which were controlled with valproic acid. Her frequent night-time parasomnias, which had first been observed around seven years of age, continued even after treatment with Depakote had been initiated. The initiation of valproic acid initially lead to developmental and behavioral improvement and especially her language improved.

She had infrequent bruxism, but never displayed any breathing abnormalities, such as hyperventilation or breath-holding (Table 1). She had intermittent constipation that was easily controlled with medicine.

MECP2 analysis and further Testing

Cranial magnetic resonance imaging (MRI) at 11 years of age showed minor tonsillar ectopia, as well as a slightly thickened corpus callosum. Two routine EEGs performed at seven and 12 years of age were normal demonstrating an age appropriate alpha-rhythm and normal background activity, while a digitrace EEG also performed at age 12 years revealed non-specific multifocal independent spike and sharp waves with no seizures. At 11 years of age KR was found to have a heterozygous c.T158M mutation, along with random XCI (70:30). This missense mutation c.T158M is found in 12.2% of cases of people with RTT (Neul et al. 2008), making it one of the most commonly found mutations linked in severity to the mid-high range of the disease. Given KR's atypical presentation, ADOS as well as ADI testing were performed at 14 years of age that indicated that she falls on the autism spectrum and has the diagnosis of Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS).

Patient MS

Developmental History—MS was born at 39 weeks after an unremarkable pregnancy and uncomplicated delivery to a healthy mother and a father diagnosed with spinal stenosis but otherwise healthy. Her initial motor development was normal: she was sitting by six months, started walking at 12 months, and had an unremarkable gait at five years. She developed a pincer grasp and the ability to finger feed her-self by one year of age. Her speech development was delayed; she acquired her first words at age four. At seven, she had a vocabulary of about 100 words and talked in phrases, often using words out of correct context and with echolalia. Her social interactions were always impaired. She displayed prominent aggressive behavior including spitting and biting. With puberty she developed OCD-like traits, including repetitive pacing, touching and arranging of toys. MS is the only person in our series that displayed any regression of skills: At eight years of age, in the context of increasing doses of antipsychotic medications prescribed for her worsening behaviors, her gait and grasp pattern started to deteriorate, as she started walking with a broad base, stumbling frequently. She also started to keep her hands fisted at baseline. She still had functional hand use however, participating in dressing herself and able to hold a cup. At 10 years of age she had completely lost her pincer grasp, and was no longer able to

hold a cup. Her active vocabulary started to decline by age 10 and a half years. But it is not until the age of 12 and a half years that she was noted to have developed increased tone at the ankles, foot posturing and toe walking and her grasp had transformed entirely to a palmar grasp when reaching for and holding on to objects and she had also lost her verbal communication.

At five years she had developed stereotypic hand movements consisting of hand flapping, mouthing, and finger wagging that were pronounced when excited or frustrated. She also developed a persistent fine tremor and truncal titubation. MS lost toilet skills after a diarrheal illness at the age of three, and suffered from frequent constipation. Breathing abnormalities with frequent breath-holding were first noted at seven years of age, and by 11 years of age she had developed prominent bruxism. Her extremities were often cold and her feet at times blue. She also developed brief periods of blotchiness in the face that were presumed to be signs of autonomic dysfunction. She was noted to be somewhat insensitive to pain and X-ray noted scoliosis of seven degrees at seven years of age. Interestingly, at two and a half years of age she had developed parasomnias with sudden awakenings and at 11 and a half years she developed episodes of behavioral arrest and staring accompanied by a left facial droop, during which she was not able to speak. They were presumed to be seizures, and oxcarbazepine was started with subsequent improvement in these episodes with dosage adjustments.

Testing—Sequence analysis of the *MECP2* gene at seven years of age revealed a c.R306H disease-causing mutation and her XCI pattern was found to be random at 70:30. A similar missense mutation c.R306C is found in 8.6% of RTT cases, belongs to the eight most common mutations found in RTT, and is associated with a milder phenotype on the spectrum, but only about 10% of these people do have any active vocabulary (Neul et al. 2008). c.R306H mutations have been described with a very similar phenotype.

A MRI brain performed at seven years of age demonstrated a small (5-6mm) hyperintense lesion in the subcortical white matter in her left parietal lobe that remained stable on repeat imaging. An EEG performed at five years showed mild slowing of the occipital dominant rhythm of 7Hz as well as paroxysmal bifrontal 6-7Hz activity, and a repeat at 11 and a half years was abnormal, showing nonspecific intermittent and at times sustained slow theta activity in all regions, along with a poorly defined 8Hz occipital rhythm, but no focal features or epileptiform activity. Formal evaluation for autism with ADOS and ADI at 9 years of age found her to meet and exceed all criteria, and she was ultimately diagnosed with having autism with an associated *MECP2* mutation, due to very poor severity scores in the domains of communication/language and social interactions (please also refer to Table 2).

Patient SB

Developmental History—Patient SB was the first child of a 26 years old mother, born at 38 weeks by induced labor for maternal pre-eclampsia. Her mother had type II diabetes mellitus and took insulin during her pregnancy. Her initial motor development was normal: she was sitting by five months of age, crawled at 10 months, and walked at 15 months. At eight months she had started feeding herself, and acquired a pincer grasp. By four years she was able to dress and undress herself, as well as turn doorknobs and open doors, and she was able to walk up and down stairs and run well.

By seven months she said “bye”, by 12 months accompanied this with an appropriate gesture, and by 15 months had a 15-word vocabulary. Her further language development was delayed however, in that at four years she was noted to have receptive and expressive language skills of a 20-months-old. Her social behavior was always very restricted. At four years she engaged only in parallel play, and her play with toys was repetitive. She did not

respond to being called by name, did not communicate verbally, nor engage in meaningful eye contact. She was unable to follow even simple verbal commands. SB also displayed some repetitive hand movements consisting of rubbing her fingertips with her thumb when excited, biting herself, or picking at her skin. She had some minor breath-holding episodes, but no hyperventilation, bruxism or drooling. She displayed none of the other remaining supportive features of variant RTT (Table 1).

Testing—A MRI of the brain obtained at 2 years of age was normal. SB developed shakiness in her movements and fine tremors especially upon awakening from sleep, and when tested for seizure activity by EEG at age 3 ½ years, it demonstrated a focus of spike activity in the right temporal region, but no seizures and was otherwise unremarkable. As SB had shown peculiarities in communication, social interests, behaviors and play interests, she was evaluated for autism by ADOS and ADI at 3 years and 7 months and was given a diagnosis of autism due to severe deficits in communication and social interactions (please see Table 2 for more detail). *MECP2*-analysis was subsequently performed at 4 years of age and revealed a c.1164-1207 deletion, and random XCI. However, she did not meet the criteria for RTT. C-terminal truncations are also common and occur in 6.9% of Rett patients and their clinical severity score typically falls on the lower end of the severity spectrum (Neul et al. 2008).

Patient AK

Developmental History—Patient AK was the first child of healthy parents that are first cousins, but do not carry a *MECP2* mutation. AK was born at 37 weeks, after an unremarkable pregnancy by emergent Cesarean section for failure to progress. She was healthy after delivery.

AK sat independently at nine months and started walking at 14 months. Subsequently, her gait remained clumsy and she had difficulties with modulating her force using either too much or too little. She held a bottle at eight months and was able to finger feed herself at 12 months. However, her grasp pattern remained immature. AK had severe delays in her language and articulation: though she started saying her first words at 10 months, she had not progressed beyond single-word phrases by the age of two-and-a-half. She started talking in sentences only by five years of age. Her language was very repetitive. Socially however, she was engaging with good eye contact.

AK was always very anxious and obsessive: she often remained fixed on certain ideas or objects; she would obsessively pull at her socks or her ears, and was perseverative in her speech. She was also noted to easily become fixated on certain ideas or objects (and mother reported that this fixation sometimes will lead to anxiety). AK had initial repetitive hand-clapping motions that subsided at around six years of age, but she continued to have hand-flapping and arm stiffening episodes when excited. Despite remaining severely developmentally delayed no signs of developmental regression were ever noted. Apart from a slight insensitivity to pain, AK had no other supportive features of variant RTT. (Table 1).

Testing—Sequence analysis of the *MECP2* gene at six years eight months revealed a c. 1164_1184 indel disease-causing mutation with random X-chromosome inactivation. This same mutation has been described before in a six-year-old girl with a mild phenotype (Corbani et al. 2012). Our patient however, did not fulfill criteria for Rett. At age 7y3mo she was assessed with the Leiter International Performance Scale - Revised and was found to be below the average range in global IQ (3rd Percentile). On formal evaluation at eight years and two months, which included ADOS/ADI, she did not satisfy the criteria for autism as she proved to be a very social child often initiating social interactions, using appropriate eye

contact which she maintains for stretches longer than 20 seconds at a time, and directing facial expressions appropriately. Also, with regard to her speech, despite significant delays, her social language was noted to be a relative area of strength. She was diagnosed largely due to her unusual interests and obsessive behavior to have Obsessive Compulsive Disorder (OCD) and Attention Deficit Hyperactivity Disorder (ADHD), apart from her Global Developmental Delay (GDD) (Table 2).

Discussion

In this report, we describe four individuals who were found to have mutations in the *MECP2* gene that are commonly encountered in patients who have RTT (Neul et al. 2008). Their ages at the last assessment span a wide range between four and twenty years. Surprisingly, three of four of these subjects have neither classical nor atypical RTT, but rather carry clinical diagnoses of autism, PDD NOS, ADHD, and OCD. The fourth did not show any features of RTT until very late, carrying a diagnosis of autism until she began losing hand skills at 8 years old at which time she started being considered to have a very mild variant of RTT, *forme fruste*. In this report we further extend the spectrum of *MECP2* associated phenotypes by presenting a case with global developmental delay with autism-associated features and OCD/ADHD.

Alternate phenotypes associated with mutations in *MECP2* have previously been described in a several reports, with autism, “Angelman-like” syndrome, and mental retardation amongst them (Harvey et al. 2007; Carney et al. 2003; Watson et al. 2001; Beyer et al. 2002; Kleefstra et al. 2004). *MECP2* mutations appear not to be a common reason to develop these disorders and the question of whether to promote routine screening for *MECP2* mutations in such conditions is still a matter of debate (Ylisaukko-Oja et al. 2005). Conversely, as mutations in the *MECP2* gene are not found in all clinical cases of RTT (mutations are found in 95% of typical RTT cases and 73.2% of atypical RTT (Neul et al. 2010)), it appears that a mutation in the *MECP2* gene is not necessary to develop Rett syndrome. Finally, the prevalence of *MECP2* mutations in neurologically normal populations is currently unknown because *MECP2* mutations do not necessarily lead to clinical features (Shevell et al. 2003).

The patients we describe here have well-established, RTT-causing *MECP2* mutations but distinctly lack key diagnostic features of RTT, including regression characteristic for Rett syndrome. The mutations that KR, MS, and SB carry have frequently been reported as causal of typical and atypical RTT. AK has the same novel complex insertion/deletion resulting in a frameshift mutation as a case classified as clinically RTT that was recently reported in the literature (Corbani et al. 2012). It is likely that the case reported in that paper is the same person as we report here, as our patient also is of Lebanese decent being followed in Canada and the US for evaluation for Rett syndrome, and as it is unlikely that two Lebanese individuals of the same age would have the exact and quite complex insertion/deletion mutation. Corbani and colleagues however, describe this person as having RTT, whereas we found that she did not have defining clinical features of RTT, and importantly did not lose any skills. As we personally evaluated AK on multiple occasions, we feel confident in our clinical assessment that she does not have RTT.

This strengthens our point that there are people with RTT disease-causing mutations (e.g. p.T158M which is one of the most commonly reported mutation in patients with RTT) who never develop RTT, but other neurodevelopmental syndromes. It also emphasizes the importance of the need to clinically evaluate patients rather than relying on genetic requisition reports alone for diagnosis. Interestingly, these four cases exhibited autistic traits to a varying degree and thus were either diagnosed with autism or an autism-spectrum disorder, with the exclusion of patient AK. Her strong social interests and interactions were

felt to be prohibitive of a diagnosis of autism, despite the fact that she demonstrated other ASD features such as insistence of sameness and repetitive behaviors. This emphasizes the role of *MECP2* in mediating core phenotypes of autism(Neul 2012; Kaufmann et al. 2011), and being in accord with previous studies demonstrating ASD traits are often found in girls that ultimately get diagnosed with RTT(Young et al. 2008).

The key reason why three of these individuals do not have the diagnosis of RTT is that they do not have a period of regression followed by stabilization or recovery, the clinical feature noted in the recent consensus criteria to be absolutely required for both typical and atypical RTT. Three out of four of our patients did not demonstrate *any* regression, but rather a delayed or fitful progression of development. This stands in contrast to the well-documented "late" regression and development of stereotypies reported in a subgroup of Rett patients with C terminal deletions (Bebbington et al. 2008). The authors also note that one patient carrying such a C-terminal deletion (patient SB) was evaluated at an early age and followed until she was about 5 years old, such that some development of RTT-associated features later in her life remains a possibility, though at this point she does not portray *any* of the necessary diagnostic and supporting criteria to support the notion that she might have an underlying variant form of RTT that might become apparent later in life.

One person (MS), who was not given the diagnosis of RTT when first examined at six years old, eventually showed some subtle motor regression at eight years old. She continued to show progressive loss of skills past 12 years old. A serious complication in this person is the need for very high doses of antipsychotic medications prior to the motor decline. Some suspicion exists that the motor decline resulted from or was exacerbated by these treatments. The overall clinical course of the loss of these skills was in marked contrast to the regression seen in both typical and atypical RTT, with retained ambulation and spoken works at age 12. Ultimately, MS is likely a case of *forme fruste* RTT. Nonetheless, the very unusual presentation and very late and mild loss of skills makes it worthwhile to note as this presentation may not usually arouse suspicion that a *MECP2* mutation is present.

It has been argued that RTT and PDD exhibit common early phenotypes of disrupted language and social development and acquisition of idiosyncratic hand movements. Specifically, in a recent paper (Young et al. 2008), it was pointed out that girls with either a p.R306C or a p.T158M mutation were more likely to initially be diagnosed with autism before eventually developing other features of RTT by three years of age. In the cases reported here, their relatively advanced ages (four, eight, 12, and 16 years) seem to preclude the further development of RTT features.

Although amongst people with typical RTT there exists a large variation in clinical presentation (Bebbington et al. 2010; Huppke et al. 2003; Shahbazian and Zoghbi 2001; Neul et al. 2008), the cases presented here show clinical presentation and disease course markedly different from RTT. These findings reinforce the notion that *MECP2* mutations by themselves are neither necessary nor sufficient to cause RTT. While a limitation of this work is that it is based on cases that presented to the Blue Bird Circle Rett Clinic at Texas Children's Hospital and thus are not population based, currently there is no population-wide testing of girls for mutations in *MECP2* which makes it impossible to do a population based study. Skewed X-chromosome inactivation can account for some of the clinical heterogeneity in patients with *MECP2* mutations, and differential symptoms could derive from the brain regions with the most mutant neurons and therefore the most dysfunction. It is unknown if results on peripheral XCI screening exactly correspond to XCI in the brain in our cases, however from the literature (Bittel et al. 2008; Gibson et al. 2005) it seems reasonable to assume that at least in most cases peripheral XCI closely resembles skewing in different brain areas, a fact that likely arises from XCI occurring at very early stages of

embryonic development. Thus, the fact that none of the cases presented here have skewed XCI in peripheral blood makes it likely that an alternate reason must exist for why the people presented here have classic disease-causing mutations in *MECP* but do not have RTT.

Thus, a significant question remains: What is the source of the clinical variation in the cases presented here? One likely source is the presence of DNA sequence changes in other loci that act as genetic modifiers to partially ameliorate the clinical phenotypes expected in people with these disease-causing mutations. A challenge will be identifying these genetic changes and verifying that they are able to modify the phenotype. Although technically difficult, advances in genetic technology make such a project feasible. The great hope is that the discovery of such genetic modifiers will allow greater understanding of the pathophysiology of RTT and MeCP2 dysfunction, and potentially the development of novel therapies.

RTT thus must remain a clinical diagnosis. The recent revision of consensus criteria (Neul et al. 2010), its confirmation in Percy et al (Percy et al. 2010), and bolstered by our findings, clearly underline this point. It is notable that *MECP2* status is not included in the criteria for diagnosis. However, given the strong association of *MECP2* mutations with developmental disorders, it is warranted to consider testing for *MECP2* mutations in girls and women with moderate to severe developmental delay (see also current AAN practice parameters (Shevell et al. 2003)). All the cases presented here were identified by primary physicians who were trying to identify the etiology of their patients' developmental delay. It is likely that many more people with similar clinical features and MECP2 mutations exist, but without greater recognition it is difficult to adequately predict who should be screened for these mutations. Finally, we reiterate that people with a neurodevelopmental disorder who are found to have mutations in *MECP2* should not automatically be given the diagnosis of RTT but should instead be described as having autism/PDD-NOS/OCD/etc. “WITH a *MECP2* associated mutation”.

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Table 1
RTT Criteria in four people with RTT mutations

Patient	**Patient KR**	**Patient MS**	**Patient SB**	**Patient AK**
Diagnosis	PDD-NOS	Autism (*forme fruste* RTT)	Autism	GDD, OCD, ADHD
Mutation	p.T158M	p.R306H	p. 1164-1207 del	c.1164_1184 del ins
X-inactivation	Random	Random	Random	Random
Period of Regression followed by recovery	No	None early, late loss of skills	No	No
Deceleration of head growth	No	No	No	No
Main Criteria				
Loss of acquired purposeful hand use	No	Yes, late (8-10yo) loss of pincer	No	No
Loss of acquired spoken language	No	Yes, late (11yo) diminished language	No	No
Gait abnormalities: dyspraxic or absent gait	No, only early on	Yes, broad based gait, dyspraxic with retropulsion (8-12yo)	No	Yes, balance problem with acceleration or stopping
Hand stereotypies	Occasional hand flapping	Hand wringing, hand to chin	Rubbed her fingertips, skin picking	Clapping of hands (resolved), flapping
Supportive Criteria for atypical RTT				
Breathing disturbances	No	Yes, periodic breathing	Few breath-holding episodes	No
Bruxism	Infrequent	Yes	No	No
Impaired sleep pattern	No	Yes at age 2.5 yo	No	No
Abnormal muscle tone	No	Increased tone at ankles	No	Slight increase at ankles
Peripheral vasomotor disturbances	Yes	Infrequent	No	No
Scoliosis/Kyphosis	No	Yes, 7 degrees	No	No
Growth retardation	No	No	No	No
Small and cold hands and feet	Cool hands and feet	Infrequently has cold hands and feet	No	No
Inappropriate screaming/laughing	No	No	No	No
Diminished response to pain	Yes (though less prominent over time)	Yes	No	Slight
Intense eye communication	No	No	No	No

Criteria required for classical RTT are a period of regression followed by recovery or stabilization, all of the main criteria and exclusion of brain injury, and grossly abnormal psychomotor development in the first 6 months.

Criteria required for the diagnosis of atypical RTT are a period of regression followed by recovery or stabilization, at least 2 of 4 main criteria, and 5 out of 11 supportive criteria.

Table 2

Diagnostic testing performed

Test	Patient KR	Patient MS	Patient SB	Patient AK
Last evaluation	20yo	16.5 yo	4 yo	8yo
MRI	Macrocephaly, cerebellar tonsillar ectopia, slightly thickened corpus callosum, no migrational abnormality	Small stable hyperintense left parietal lobe subcortical white matter lesion	Normal	Not done
EEG	EEG within range of normal variation. (7&12yo)DIGITRACE:independent multifocal spikes and sharp waves (12yo)	Intermittent and at times sustained slow theta activity in all regions. (11yo)	Normal at 2yo.spike focus in the right temporal area (3.5 yo)	Not done
ADOS	Met criteria for ASD:	Met criteria for Autism:	Met criteria for ASD:	Did *NOT* fulfill criteria for Autism/ASD:
ADOS modules	Module 2	Module 2	Module 1	Module 2
Age	14yo	9yo	3y7months	8yo
Severity – Scores	4,4,2,0	8,10,2,5		
Com.	Social overtures and responses diminished and awkward. Verbalizations unusual but not autistic, no echolalia; responded to simple questions.	Mostly single words (∼100), occasional longer utterances, some stereotypic.	No eye contact, facial expression, gestures or pointing. No directed vocalizations.	Often initiated social interactions, used eye contact, initiated joint attention, directed facial expressions. Uses language appropriately, with short phrases predominantly.
	Frequent eye contact with mother, appropriate with examiner. Initiated joint attention.	Speech monotone, slowed pace and articulation. Points without eye contact; eye contact only staring, face typically bland, bursts of smiling at random.		
Soc.	Multiple social overtures to mother and occasional toward examiner.	Little social communication, few to no social overtures with examiner,	Likes being near people yet does not know how to pursue social interactions, no reciprocity in interactions; does not respond to name.	Very social child, interested in interacting – some difficulties with reciprocal social interactions and conversations.
Imag.	Very little imaginative play and no functional play, little engagement in birthday party scenario, attempted to model use of dolls.	No functional or imaginative play	Limited reciprocal and imaginary play.	Limited imagination/creativity.
Ster.	No unusual sensory interests, no unusual motor mannerisms, no repetitive interests.	Repeat posturing and gazing at self. Thumbs often fisted and brought to mouth pressed against	No unusual hand movements.	Has unusual sensory interests, unusual hand and finger movements, gestures, and repetitive interests.
ADI				

Test	Patient KR	Patient MS	Patient SB	Patient AK
Age	14yo	9yo	3y7months	8yo
Interpretation	Met and exceeded scores on all four possible categories for autism spectrum disorder.	Met and exceeded the scores that indicate diagnosis in all four possible categories.	Met criteria for diagnosis of autism.	Did not satisfy the criteria for autism.
Additional Testing	Gilliam Autism Rating Scale (11y10mo): Stereotyped Behavior - 10 Social Interaction - 14 Developmental - 16 OVERALL: 121	None	PEP-R (3y7mo): Overall 15 to 18 month equivalent.	CONNORS BASC-II: Consistent with ADHD - combined type Leiter-R (7y3mo):Global IQ - below average score at 3rd percentile