

Rett Syndrome in Females With CTS Hot Spot Deletions: A Disorder Profile

E. Smeets,^{1,2*} P. Terhal,³ P. Casaer,⁴ A. Peters,⁵ A. Midro,⁶ E. Schollen,¹ K. van Roozendaal,² U. Moog,² G. Matthijs,¹ J. Herbergs,² H. Smeets,² L. Curfs,^{2,7} C. Schrandt-Stumpel,^{2,7} and J.P. Fryns^{1,2}

¹Centre of Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium

²Department of Clinical Genetics, Academic Hospital Maastricht, Maastricht, The Netherlands

³Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands

⁴Department of Pediatrics, University Hospital Gasthuisberg, Leuven, Belgium

⁵Department of Neurology, University Medical Center Utrecht, Utrecht, The Netherlands

⁶Department of Human Genetics, Medical University of Bialystok, Bialystok, Poland

⁷Research Institute Growth and Development, Maastricht University, Maastricht, The Netherlands

From a series of 107 females with Rett syndrome (RTT), we describe the long-term history of ten females with a deletion in the C-terminus of the *MECP2* gene. We observed that their disorder profile is clinically recognizable with time and different from other atypical and milder RTT phenotypes. In females with hot spot deletions in the C-terminus, dystonia is present from childhood and results in a serious spine deformation in spite of preventive measures. Their adaptive behavior is surprisingly better preserved and in contrast with the typical decline in motor functioning. The delineation of disorder profiles by long-term clinical observation can teach us about genotype/phenotype relationships and eventually about the effect of epigenetic phenomena on the final phenotype. © 2004 Wiley-Liss, Inc.

INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder [Hagberg et al., 1983] caused by a mutation in the gene encoding methyl-CpG-binding protein 2 (*MECP2*) [Amir et al., 1999]. Classical and variant phenotypes are diagnosed clinically according to international criteria [Hagberg et al., 1990, 2002]. Literature data show that mutations are found in more than 80% of RTT cases, with the highest mutation rates in classical RTT and in about 65% of the variants [Smeets et al., 2003]. More than 200 different mutations have been described in RTT and about 10% consist of intragenic deletions in the C-terminal segment (CTS) of the *MECP2*-gene, between nucleotides 1,050 and 1,200 [Lee et al., 2001]. The aim of the present study was to evaluate the long-term history of ten females with deletions in this hot spot of the gene with respect to preservation of motor abilities, communication, and behavior.

MATERIALS AND METHODS

Between 1983 and 2002, we examined 107 girls and women, aged between 2 and 61 years, with a clinical diagnosis of Rett

syndrome. According to the revised clinical criteria for diagnosis of classical and variant phenotypes [Hagberg et al., 2002] 64 (60%) presented the classical phenotype (CR) and 43 (40%) one of the RTT variants (RV). We used the clinical scoring system as described by expert clinicians [Kerr et al., 2001] in all females. Each RTT individual was observed for personal abilities and behavioral characteristics and assessed by the first author together with the parents and/or caretakers. A second more simplified score was given leaving out sleep disorder and mood disturbance, breathing dysrhythmia, muscle tone, involuntary movements, head circumference and other growth parameters. In these milder RTT variants these manifestations were not markedly present and not contributing to the clinical severity. Gross motor sitting, and walking ability, remaining functional hand use, speech ability, epilepsy, and neurogenic scoliosis were, therefore, considered more likely to influence the long term evolution. A score from 0 to 3 was given for these six items as shown in Table I. 0 for the normal situation; 1 when there was impairment without total loss of motor ability, reduced hand use, some preserved speech, previous epilepsy, or seizures well controlled by medication, mild scoliosis; 2 when there was loss of function, apraxia, no speech, uncontrolled epilepsy, severe scoliosis; 3 when the function was never acquired, status epilepticus, surgery for scoliosis. The maximum score is 18: below 9 the condition was considered as mild.

DNA analysis of the *MECP2* gene was performed by DHPLC screening followed by sequencing analysis to identify and confirm the nature of the mutation. Exon 3 and 4 were amplified by a standard touch down PCR from genomic DNA in respectively two and five overlapping fragments, essentially as described by Amir et al. [1999]. The fragments were analyzed by DHPLC (WAVE system, Transgenomic, Crewe, UK). Possible mutations were subsequently identified by sequencing with Big Dye Terminators as described by the manufacturer (Applied Biosystems). In the initially mutation-negative subjects, analysis was expanded by complete sequencing and LR PCR. Finally, a Southern Blot and MLPA were performed to establish larger deletions or gross rearrangements as described by Schollen et al. [2003]. X-inactivation was studied in 24 of the 107 females. Tested X-inactivation patterns were skewed in five but could not be related to the degree of clinical severity. The overall mutation detection rate in the whole series was 87%, 92% in CR, and 79% in RV. Recurrent mutations were found as well as complex mutations and gross rearrangements [Schollen et al., 2003]. We found ten variants (9%) with deletions in the hot spot of the CTS, all detected by DHPLC screening. Cases 1, 5, 6, 7, and 8 were previously described [Smeets et al., 2003].

*Correspondence to: E. Smeets, M.D., Academic Hospital Maastricht, Department of Clinical Genetics, P.O. Box 1475, 6201 Maastricht, The Netherlands.
E-mail: eric.smeets@gen.unimaas.nl

Received 17 March 2004; Accepted 11 August 2004

DOI 10.1002/ajmg.a.30410

TABLE I. Simplified Score System

Score	0	1	2	3
Sitting	Normal	Impaired	Lost	Never acquired
Walking		Impaired	Lost	Never acquired
Hand use		Reduced	Lost	Never acquired
Speech		Some words	Lost	Never acquired
Epilepsy		Controlled	Uncontrolled	Status epilepticus
Scoliosis/kyphosis		Mild	Severe	Operated

RESULTS

Table II presents the individual data of the ten females, the molecular findings and simplified score at the time of the last examination. Retrospectively, the age at diagnosis was between 3 and 13 years in nine individuals; individual five was diagnosed at the age of 54 years.

All ten patients were able to sit. Walking was considered impaired in patients 4, 7, and 10. In patient 7, imbalance was due to anxiety after the operation for scoliosis and residual pain. Walking patterns, apart from being broadly based in all cases were particularly awkward in patient 4 and 10. They used their left leg to choose direction of gait tilting the other leg very high up before placing it back onto the floor causing them to walk in circles. Patient 9 (Fig. 1), the mildest in clinical presentation, took short stiff steps like in patients with Parkinson disease (walking behind the gravity point). She needed orthopedic shoes with an upgrading under her forefoot to help her stop in time at the target. Hand use was reduced in nine patients, and totally absent in patient 4. Hands were used in automatic handling (e.g., opening doors, clothing, shake hands, self-feeding, and drinking) as well as in intentional grasping. Intentional grasping is clearly present in most cases, but the reaching out is suddenly interrupted by flexion of the elbow and lost into the characteristic stereotyped movement. The preserved level of grasping, fine motor pincer grasp or gross motor tripod grasp, mimics the grasping patterns of young infants. Hands, fingers (and even feet) were also used in contact behavior for touching, caressing, pitching, and skin picking. Speech was present in cases 1, 6, and 7 with the use of several meaningful words or two word phrases in direct communication. This ability seems to fluctuate with age. Parents indicated in cases 1 and 6 that this ability was declining, and in case 7 regaining with advancing age. Epilepsy was uncontrolled by medication in patient 2. The remaining patients had been seizure free for many years or were well controlled by medication. The most remarkable manifestation in this group was their spine deformation. The scoliosis (cases 1, 2, 4, 6, 7, 8) was S-shaped and dextro-sinistro convex. It interfered with

walking and pulmonary function necessitating surgical intervention in four patients in adolescence. In patient 2, parents refused surgery because of poor nutritional condition. Patient 6 had a severe but stable scoliosis at the time of examination at age 25 years. In the four patients (3, 5, 9, 10) with a high kyphosis the bending with protruding shoulders started in childhood and progressed in adolescence. The associated scoliosis was of the low lumbar torsion type.

Dystonia (or extrapyramidal asymmetry) is a well known manifestation of RTT. In these individuals it is probably the major cause of the spine deformation since hypotonia and muscle wasting were not present at the time of examination. The females with kyphosis might have had axial hypotonia; those with scoliosis more extrapyramidal asymmetry.

Table III comments on their personal history, abilities, and behavior. All females presented as RTT variant in infancy except for patient 5 who presented with psychomotor retardation from birth on. She was diagnosed as congenital onset variant but without devastating early epileptic encephalopathy. The behavior of these ten females can be defined as lively, curious, alert, friendly, tempting, and teasing at younger age, and prone to agitation and crying when they feel not safe. Some remain often "in their own world" giving the impression of "autism." All of them used their eyes to express feelings, wishes, and needs in the for RTT characteristic way. Many remain able to learn about new situations and persons in their daily surrounding. But in spite of a relatively well-preserved adaptive behavior they became more passive in behavior with lesser facial expression with advancing age.

DISCUSSION

We report on ten RTT females with an intragenic deletion in the C-terminal segment (CTS) of the *MECP2* gene. Although they present as "classical" at older age, in the beginning they are like the form fruste as described by Hagberg et al. [1993, 1994]. The course of the disorder in all of them is more protracted in time, with more preserved cognitive functions in

TABLE II. Clinical and Molecular Data of 10 Females With CTS Hot Spot Deletions

Case	YOB	Nucleotide change	XCI	Onset (month)	Diagnosis (year)	Examined (year)	Score	Sitting	Walking	Hand use	Speech	Epilepsy	Scoliosis
1	1977	1151del41	rd	17	3	23	6	0	0	1	1	1	3
2	1988	1152del41		14	5	15	7	0	0	1	2	2	2
3	1989	1157del44		14	13	13	4	0	0	1	2	0	1
4	1976	1159del35		18	13	26	8	0	1	2	2	0	3
5	1946	1163del45	rd	2	54	55	6	0	0	1	2	1	2
6	1976	1164del44	rd	12	10	25	5	0	0	1	1	1	2
7	1982	1164del44	rd	15	2	19	7	0	1	1	1	1	3
8	1987	1164del44	skw	12	4	14	6	0	0	1	2	1	3
9	1989	1226del41		18	11	13	3	0	0	1	2	0	0
10	1985	1232del44		12	9	17	6	0	1	1	2	1	1

XCI, X inactivation; rd, random; skw, skewed.



Fig. 1. Patient 9 at age 13 years.

adolescence and adulthood. Their main clinical problem is a gradual decline of gross motor ability in spite of all preventive measurements, and a rapidly progressive spine deformation as a consequence of marked dystonia that is present from childhood. With advancing age they become more impaired in walking, at least slower and more passive in general motor performance, more inappropriate in hand use and with the

tendency to use lesser verbal expression (in the preserved speech variants). The short stature (as expected in classical RTT), the overall rigid posturing with pronounced kyphosis or scoliosis, the abiotrophic changes in skin and under lying muscles and the slowing of motor performance, give them a “pre-aging” appearance. In contrast with this decline in motor capacities is the preservation of simple communicative and

TABLE III. Clinical Comments on Disorder Profile

Case	
1	PSV, rapid progressive scoliosis (operation at 16 years), some preserved speech, tripodgrasp, friendly quiet behavior, uses eye contact to express feelings, wishes, and needs
2	FF as a child, more and more classical as an adult: cluster epilepsy, severe S-scoliosis not operated, poor nutritional condition and muscle wasting, no mood swings, no breathing irregularities, truncal ataxia progressing with age, dyspraxic reduced hand use but very agile tripod grasp, quiet behavior, sometimes in her own world, uses eye contact to express feelings, wishes, and needs, (sudden death in 2003)
3	FF, high kyphosis, lumbar scoliosis, indicates bladder control, no mood disturbance, good general condition, predominant autistic behavior, refuses contact when not on her own initiative
4	FF, progressive scoliosis since age 8 years (operated at 19 years), periodic involuntary and cloniform jerking of the left leg at the age of 26 years, “leftfooted,” feels with the left foot, quiet behavior as an adult, spells of agitation and automutilation in childhood, still mood swings
5	COV, early onset of retardation, severe kyphosis and scoliosis, hands on midline but opens doors, friendly but yells when feels not safe, knows her way in the group home, recognizes persons, helps herself in daytime activity, severe equinus of feet, asymmetric hypertonia, contractures of fingerjoints, likes body care, painted fingernails, and hairdresser
6	PSV, rapid progressive scoliosis from 10 years of age, some words and reduced hand use, normal behavior, lost some vocabulary the last years
7	PSV, rapid progressive scoliosis (posterior and anterior fusion at 19 years), reduced motor performance from 16 years, friendly, social, and alert, two word phrases, still learning new things and regaining more words in the last years, recognition of persons, refuses to walk after the scoliosis operation
8	FF, rapid progressive scoliosis (operation at 14 years), panting and bloating, knows SMOG pictograms, friendly, social, and alert, seeking contact by touching and pinching or putting her hand on your shoulder, uses eye contact to express feelings, wishes, and needs
9	FF, high kyphosis with protruding shoulders, speech is more a directed imitation of words and sounds, short attention span in play and contact with non-verbal developmental level between 12 and 15 months, still able to learn new things, makes drawings, knowledge of concepts likes colors and cars, intermediate pincer to tripod grasp, mild gross tremor, grimacing facial expression, no mood swings, friendly teasing behavior, sometimes in her own world, easily expressing wishes, and needs, recognition of persons and pictograms
10	FF, marked kyphosis with protruding shoulders when seated, panting, bloating, uses eye contact to express feelings, wishes, and needs, points left-handed, reduced tripod hand use, does not like to be touched, prone to agitation when not safe, “leftfooted” gait and awkward tilting of the right leg, mild gross tremor

PSV, preserved speech variant; FF, form fruste; COV, congenital onset variant.

cognitive abilities, even still able to recognize and to learn about new persons and situations in their daily surrounding far into adult life. Recently a case report of an odd RTT variant with a CTS hot spot deletion [Hagberg et al., 2003] was described. Her history and disorder profile seems very similar to the patients described in the present report. Hagberg et al. [2003] refers to the ongoing neuromotor impairment as a decline “at the output-side” in contrast to a better functioning “at the input-side.”

Describing disorder profiles, obtained through long-term observation can teach us about the genotype/phenotype relationship and might especially contribute to the understanding of modifying factors [Renieri et al., 2003] or epigenetic phenomena influencing the expression of mutations [Chen et al., 2003; Martinowich et al., 2003]. XCI patterns were not contributing to the disorder profile but were not studied in all cases. In our series the CTS hot spot deletions represent 9% of the RTT genotypes. This confirms that they form a major group in the spectrum of recurrent mutations [Laccone et al., 2001; Lee et al., 2001]. Some authors have correlated the type and the location of mutations with the clinical phenotype [Huppke et al., 2002; Leonard et al., 2003]. They concluded that all mutations that lead to either complete or partial truncation of the region encoding for the nuclear localization signal are associated with a more severe phenotype than other truncating mutations. In addition there is evidence that the carboxyl-terminal segment of MeCP2 facilitates binding to the nucleosome core [Chandler et al., 1999]. In CTS hot spot deletions truncation occurs beyond the coding regions and is not involving the nuclear localization signal. This might partially explain their atypical or “milder” course that is however in time still clinically recognizable as Rett syndrome.

CONCLUSIONS

In our experience, the CTS hot spot deletion clinically leads to a disorder profile that is recognizable in time and different from other atypical and milder RTT phenotypes. In females with CTS hot spot deletions dystonia produces more rapidly, from pre-school age on, a serious spine deformation in spite of all preventive measures. Their adaptive behavior is surprisingly better preserved and in contrast with a gradual decline in motor performance.

Grouping individual patients by location of their mutation in the *MECP2* gene rather than by mutation type will contribute to the actual knowledge about the genotype-phenotype relationship. A long-term follow up is important with respect to the development of therapies or rehabilitation measurements to eventually prevent further impairment.

REFERENCES

- Amir R, Van den Veyver I, Wan M, Tran C, Francke U, Zoghbi H. 1999. Rett Syndrome is caused by mutations in X-linked *LMCP2*, encoding methyl-CpG-binding protein 2. *Nat Genet* 23:185–188.
- Chandler SP, Guschin D, Landsberger N, Wolffe AP. 1999. The methyl-CpG binding transcriptional repressor MeCP2 stably associates with nucleosomal DNA. *Biochemistry* 38(22):7008–7018.
- Chen W, Chang Q, Lin Y, Meissner A, West A, Griffith E, Jaenisch R, Greenberg M. 2003. Depression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* 302(5646):885.
- Hagberg B, Gilberg C. 1993. Rett variants-Rettoid types. In: *Rett syndrome, clinical and biological aspects*. Clinics in Developmental Medicine. Vol. 127. Cambridge: MacKeith Cambridge University Press. pp 40–60.
- Hagberg B, Skjeldal O. 1994. Rett variants: A suggested model for inclusion criteria. *Pediatr Neurol* 11:5–11.
- Hagberg B, Aicardi J, Dias K, Ramos O. 1983. A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Ann Neurol* 14:471–479.
- Hagberg B, Goutieres F, Hanefeld F, Rett A, Wilson J. 1990. Rett syndrome: Criteria for inclusion and exclusion. *Brain Dev* 12:47–48.
- Hagberg B, Hanefeld F, Percy A, Skjeldal O. 2002. An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett syndrome clinical consensus panel satellite to European paediatric neurology society meeting, Baden Baden, Germany, 11 September 2001. *Eur J Paediatr Neurol* 6(5):293–297.
- Hagberg B, Erlandsson A, Kyllerman M, Larsson G. 2003. Odd *MECP2*-mutated Rett variant-long-term follow-up profile to age 25. *Eur J Paediatr Neurol* 7(6):417–421.
- Huppke P, Held M, Hanefeld F, Engel W, Laccone F. 2002. Influence of mutation type and location on phenotype in 123 patients with Rett syndrome. *Neuropediatrics* 33(2):63–68.
- Kerr A, Nomura Y, Armstrong D, Anvret M, Belichenko P, Budden S, Cass H, Christodoulou J, Clarke A, Ellaway C, d'Esposito M, Francke U, Hulten M, Julu P, Leonard H, Naidu S, Schanen C, Webb T, Engerstrom I, Yamashita Y, Segawa M. 2001. Guidelines for reporting clinical features in cases with *MECP2* mutations. *Brain Dev* 23:208–211.
- Laccone F, Huppke P, Hanefeld F, Meins M. 2001. Mutation spectrum in patients with Rett syndrome in the German population: Evidence of hot spot regions. *Hum Mutat* 17(3):183–190.
- Lee S, Wan M, Francke U. 2001. Spectrum of *MECP2* mutations in Rett syndrome. *Brain Dev* 23:S138–S143.
- Leonard H, Colvin L, Christodoulou J, Schiavello T, Weaving L, Williamson S, Davis MD, Ravine D, Fyfe S, de Klerk N, Matsuishi T, Kondo I, Clarke A, Hackwell S, Yamashita Y. 2003. Patients with the R133C mutation: Is their phenotype different from Rett syndrome patients with other mutations? *J Med Genet* 40(5):E53.
- Martinowich K, Hattori D, Wu H, Fouse S, He F, Fan G, Sun Y. 2003. DNA methylation-related chromatin remodeling in activity-dependent Bdnf gene regulation. *Science* 302(5646):890.
- Renieri A, Meloni I, Longo I, Ariani F, Mari F, Pescucci C, Cambi F. 2003. Rett syndrome: The complex nature of a monogenic disease. *J Mol Med* 81:346–354.
- Schollen E, Smeets E, Deflem E, Fryns JP, Matthijs G. 2003. Gross rearrangements in the *MECP2* gene in three patients with Rett syndrome: Implications for routine diagnosis of Rett syndrome. *Hum Mut* 22(2):116–120.
- Smeets E, Schollen E, Moog U, Matthijs G, Herbergs J, Smeets H, Curfs L, Schrandt-Stumpel C, Fryns JP. 2003. Rett syndrome in adolescent and adult females: Clinical and molecular genetic findings. *Am J Med Genet* 122A:227–233.