

Rett Syndrome Diagnostic Criteria: Lessons from the Natural History Study

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Analysis of 819 participants enrolled in the Rett syndrome (RTT) Natural History Study validates recently revised diagnostic criteria. 765 females fulfilled 2002 consensus criteria for classic (653/85.4%) or variant (112/14.6%) RTT. All participants classified as classic RTT fulfilled each revised main criterion; supportive criteria were not uniformly present. All variant RTT participants met at least 3 of 6 main criteria in the 2002, 2 of 4 main criteria in the current format, and 5 of 11 supportive criteria in both. This analysis underscores the critical role of main criteria for classic RTT; variant RTT requires both main and supportive criteria.

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The diagnosis of Rett syndrome (RTT), described by Andreas Rett in 1966¹ as a neurodevelopmental disorder predominantly affecting females, is based on clearly defined clinical criteria, modified periodically with improved understanding of its core features. In 1985, Hagberg and colleagues developed consensus criteria exclusively for females,² modified in 1988 to include males.³ Following identification of mutations in the methyl-CpG-binding-protein 2 (*MECP2*) in individuals with RTT,⁴ an international consensus meeting further modified those criteria to affirm the importance of carefully applied diagnostic criteria⁵ and to clarify possible ambiguities in interpretation of these criteria. This included recognition that (1) abnormal deceleration in the rate of head growth was not always present, (2) early development could be delayed, and (3) apraxia of gait should include the possibility of failure to develop gait. Further, it was important to provide consensus criteria for variant forms of RTT.⁶ More recently, RettSearch, an international group of clinicians, completed a systematic review of these criteria to provide further clarifications,

including those core features that are essential for considering the diagnosis of RTT or its variants and to refine definitions of other previous criteria not included in this core group.⁷ This revision follows on refinements in molecular diagnosis, including identification of previously unrecognized *MECP2* mutations in exon 1,⁸ large deletions encompassing 1 or more exons,^{9,10} and duplication of the *MECP2* Xq28 region in males with severe neurodevelopmental disorders,^{11–13} as well as confirmation of mutations in 95% of females with classic RTT.¹⁴ Furthermore, an increasing number of females and males who do not meet RTT criteria have been identified with *MECP2* mutations.

The RTT Rare Disease Clinical Research Center (RDCRC) natural history study began enrolling participants in 2006, with the goal of enrolling 1,000 individuals with classic or variant RTT based on the 2002 criteria.⁵ The present analysis was conducted on 819 participants enrolled as of February 28, 2010, to assess the recently modified clinical criteria. The results validate the revised diagnostic format and emphasize the importance of having clearly defined criteria based on clinical features including clear regression of previously acquired skills in both classic and variant RTT and the necessity of supportive criteria only for variant forms.

Patients and Methods

Participants were enrolled into the RDCRC natural history study if they met diagnostic criteria for RTT or had a mutation in *MECP2*. All participants had complete mutation testing, including *MECP2* sequencing and, if negative, deletion/duplication testing. Clinical diagnosis utilized the 2002 consensus criteria.⁵ Classic RTT was based on meeting all necessary criteria, although supportive criteria from the 2002 criteria were also assessed (periodic breathing, bruxism, sleep disruption,

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TABLE 1: Natural History Study: *MECP2* Status

Mutation	Classic, No. (%)	Variant, No. (%)	Non-RTT, No. (%)		NA Database (%) ¹⁵
			Female	Male	
T158M	72 (11.0)	4 (3.6)	1 (3.2)	0	11.9
R255X	71 (11.0)	10 (8.9)	1 (3.2)	0	9.0
R168X	68 (10.4)	4 (3.6)	0	0	9.4
R306C	45 (6.9)	7 (6.3)	4 (12.9)	0	6.9
R294X	41 (6.3)	4 (3.6)	1 (3.2)	0	6.2
R270X	36 (5.5)	4 (3.6)	0	0	7.2
R133C	25 (3.8)	10 (8.9)	4 (12.9)	0	6.4
R106W	20 (3.1)	2 (1.8)	1 (3.2)	1 (4.4)	4.4
Large deletion	57 (8.7)	4 (3.6)	2 (6.5)	0	6.4
C-terminal truncation	45 (6.9)	14 (12.5)	12 (38.7)	3 (13.0)	8.8
Other mutations	130 (19.9)	17 (15.2)	5 (16.2)	19 (82.6)	23.4
>1 mutation	10 (1.5)	2 (1.8)	0	0	NR
No mutation	33 (5.0)	30 (26.8)	0	0	NR
Total	653	112	31	23	1,059

RTT = Rett syndrome; NA = North American; NR = not recorded.

abnormal muscle tone, vasomotor disturbances, scoliosis, growth failure, and small hands and feet). For variant RTT, diagnosis was based on the 2002 variant criteria, namely, meeting 3 of 6 main criteria (loss of hand and communication skills, babble speech, hand stereotypies, deceleration of head growth, and a disease profile of regression followed by recovery of interaction) and 5 of 11 supportive criteria (periodic breathing, aerophagia, bruxism, apraxia of or no gait, scoliosis, lower limb muscle atrophy, cold feet, sleep disruption, inappropriate screaming/laughing, diminished nociception, and intense eye contact).

The following analyses were conducted on 819 participants enrolled through February 28, 2010: (1) diagnostic category (classic RTT, variant RTT, or non-RTT using the 2002 criteria) and distribution of *MECP2* mutations within each category and (2) number of participants in each category meeting the diagnostic and supportive criteria. This cross-sectional analysis is based on assessment at initial enrollment and does not reflect longitudinal follow-up. The clinical diagnostic categories determined using the 2002 criteria for these individuals were compared with the diagnostic categories that would be assigned to these individuals using the revised 2010 criteria.⁷

Results

The distribution of *MECP2* mutations for 819 participants enrolled as of February 28, 2010 is displayed in Table 1. Data include frequency of the 8 most common mutations, large deletions, C-terminal truncations, all

other mutations, and no mutations as well as occurrence of >1 mutation in participants with classic and variant RTT. Of 765 females fulfilling criteria for classic or variant RTT, 653 (85.4%) were diagnosed as classic, and 112 (14.6%) were classified as variant. A mutation was identified in 95% of 653 females with classic and 73.2% of 112 females with variant RTT and in all 31 females (identified either as carriers in multiplex families, based on a single feature such as hand stereotypies or deceleration of head growth, or in some female cases as having developmental delay and autistic features) and 23 males who did not meet criteria for classic or variant RTT. In classic RTT, frequency of the 8 most common mutations was similar to that in the North American database.¹⁵ The greater frequency of large deletions in the natural history study likely reflects the requirement for complete testing in the present study, as large deletion testing was not completed uniformly in North American database registrants. For variant RTT and females with non-RTT, the greater frequency of C-terminal truncations, R133C, and R306C (non-RTT females only) confirms earlier reports of milder clinical severity for these mutations.^{14,16} Males with non-RTT tend to have mutations not commonly identified in classic RTT. Although we have evaluated several potential participants with *CDKL5* mutations, only 1 participant is included in this study, because most fail to meet RTT clinical criteria.

TABLE 2: Natural History Study: Diagnostic Criteria

2002 Criteria	2010 Revised Main Criteria	Classic, No. (%)		Variant, No. (%)		Non-RTT Female, No. (%)		Non-RTT Male, No. (%)	
		Yes	No	Yes	No	Yes	No	Yes	No
Normal prenatal		644 (99)	9 (1)	97 (87)	15 (13)	28 (90)	3 (10)	22 (96)	1 (4)
Normal perinatal		643 (98)	10 (2)	99 (88)	13 (12)	30 (97)	1 (3)	22 (96)	1 (4)
Normal initial development		645 (99)	8 (1)	65 (58)	47 (42)	27 (87)	4 (13)	13 (57)	10 (43)
Loss of hand use	✓	653 (100)	0	60 (54)	52 (46)	1 (3)	30 (97)	10 (43)	13 (57)
Loss of communication	✓	653 (100)	0	63 (56)	49 (44)	6 (19)	25 (81)	11 (48)	12 (52)
Hand stereotypies	✓	653 (100)	0	110 (98)	2 (2)	9 (29)	22 (71)	16 (70)	7 (30)
Head growth deceleration		535 (82)	118 (18)	67 (60)	45 (40)	8 (26)	23 (74)	11 (48)	12 (52)
Gait apraxia/no gait	✓	653 (100)	0	104 (93)	8 (7)	13 (42)	18 (58)	19 (83)	4 (17)
No IUGR		650 (99)	3 (1)	105 (94)	7 (6)	28 (90)	3 (10)	22 (96)	1 (4)
No organomegaly		653 (100)	0	109 (97)	3 (3)	25 (81)	6 (19)	22 (96)	1 (4)
No retinopathy		653 (100)	0	108 (96)	4 (4)	27 (87)	4 (13)	22 (96)	1 (4)
No peri-/postnatal injury		653 (100)	0	108 (96)	4 (4)	26 (84)	5 (16)	21 (91)	2 (9)
Total		Classic RTT = 653		Variant RTT = 112		Non-RTT female = 31		Non-RTT male = 23	

RTT = Rett syndrome; IUGR = intrauterine growth retardation.

TABLE 3: Natural History Study: Supportive Criteria

Criteria	Classic, No. (%)		Criteria	Variant, No. (%)	
	Yes	No		Yes	No
Periodic breathing	500 (77)	153 (23)	Periodic breathing	68 (61)	44 (39)
Bruxism	595 (91)	58 (9)	Bruxism	93 (83)	19 (17)
Sleep disruption	458 (70)	195 (30)	Sleep disruption	81 (72)	31 (28)
Abnormal muscle tone	505 (77)	148 (23)	Abnormal gait	104 (93)	8 (7)
Cold hands/feet	273 (42)	380 (58)	Cold feet	21 (19)	91 (81)
Scoliosis	321 (49)	332 (51)	Scoliosis	40 (36)	72 (64)
Growth failure	454 (70)	199 (30)	Aerophagia	43 (38)	69 (62)
Small hands	152 (23)	501 (77)	Lower limb muscle atrophy	23 (21)	89 (79)
Small feet ^a	425/642 (66)	217/642 (34)	Laughing/screaming spells	70 (63)	42 (37)
			Reduced nociception	80 (71)	32 (29)
			Intense eye contact	74 (66)	38 (34)
Total	653		Total	112	

^aValues for foot length were available for 642 of 653 (98%) classic Rett syndrome participants.

Table 2 indicates the number of participants in each group fulfilling the 2002 necessary and exclusion criteria. For classic RTT, the prenatal, perinatal, and early development periods appeared normal in most participants. The small group (1–2%) with minor prenatal, perinatal, or early development abnormalities did not include any significant problem leading to neurological dysfunction. Similarly, among the exclusion criteria, the infrequent occurrence of intrauterine growth retardation (<1%) was also not associated with a pervasive systemic or neurological disorder. All participants with classic RTT fulfilled the main criteria in the 2010 revised version,⁷ namely, regression including loss of communication and fine motor hand skills, apraxia or absence of gait, and hand stereotypies. Abnormal deceleration in the rate of head growth was noted in 82%, supporting the 2002 criteria revision expecting this finding “in the majority.” None of the supportive criteria from the 2002 criteria was present in every classic RTT participant, and only bruxism was noted in >80% (Table 3). As such, supportive criteria are not crucial for diagnosis in classic RTT.

Among the variant RTT participants, all met the 2002 consensus criteria and the recently revised criteria for at least 2 of 4 main and 5 of 11 supportive criteria. Loss of hand use and communication and abnormal head growth deceleration were noted in 54 to 60%, whereas hand stereotypies appeared in 98% (see Table 2). For supportive criteria in variant RTT (see Table 3), 7 were present in >50% (abnormal gait [93%], bruxism [83%], sleep disruption [72%], reduced nociception [71%], intense eye contact [66%], inappropriate laughing/screaming [63%], and periodic breathing [61%]). Scoliosis, lower limb muscle atrophy, cold feet, and aerophagia were much less common. As such, in combination with the main criteria, supportive criteria appear essential for the diagnosis of variant RTT.

For non-RTT females, only 1 lost hand use, and other main criteria of the 2010 revision did not exceed 30%. Abnormal gait was noted in 42%. In contrast, a majority of non-RTT males had hand stereotypies, particularly repetitive rubbing of the nose, and early development was normal in only 57%. Among the non-RTT group, preserved eye contact and reduced nociception were present nearly uniformly, whereas the remaining variant supportive criteria were observed less frequently, with only bruxism and sleep disruption (52%) and abnormal gait (59%) in a majority of these participants. As the non-RTT participants do not fulfill the main criteria, application of the supportive criteria does not appear to be critical.

Discussion

Analysis of diagnostic features for 819 participants enrolled in the RDCRC natural history study, based on the 2002 diagnostic criteria, underscores the critical role of the main criteria, namely, regression of fine motor and

communication skills, abnormal or no gait, and hand stereotypies for the diagnosis of classic and variant RTT. For classic RTT, these core criteria are sufficient for diagnosis, as none of the eight 2002 supportive criteria is noted uniformly. For variant RTT, diagnosis requires application of both main (2 of 4) and supportive (5 of 11) criteria. They should be considered in partnership.

In their summation of the 2002 consensus criteria,⁵ the authors commented that “In order for the accurate conduct of phenotype-genotype studies, these criteria must be applied consistently. This seems self-evident. However, such is not always the case.” As we approach treatment trials in RTT, the importance of consistent application of diagnostic criteria is even more crucial. The present analyses validate the conceptual framework incorporated within the most recent revision of RTT diagnostic criteria, demonstrate that utilization of the revised criteria does not alter pre-existing diagnostic categorization, and underscore the importance of clearly defined criteria based on clinical features of RTT.

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Potential Conflicts of Interest

Nothing to report.

References

1. Rett A Über ein eigenartiges hirnatrophisches Syndrom bei Hyperammonämie im Kindesalter. *Wien Medizin Wochschr* 1966;116: 723–726.

2. Hagberg B, Goutieres F, Hanefeld F, et al. Rett syndrome: criteria for inclusion and exclusion. *Brain Dev* 1985;7:372–373.
3. Group TRSDCW Diagnostic criteria for Rett syndrome. *Ann Neurol* 1988;23:425–428.
4. Amir R, Van den Veyver I, Wan M, et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23:185–188.
5. Hagberg B, Hanefeld F, Percy A, et al. An update on clinically applicable diagnostic criteria in Rett syndrome. Comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, 11 September 2001. *Eur J Paediatr Neurol* 2002;6:293–297.
6. Hagberg BA, Skjeldal OH. Rett variants: a suggested model for inclusion criteria. *Pediatr Neurol* 1994;11:5–11.
7. Neul J. *Ann Neurol* 2010;68:000–000.
8. Mnatzakanian GN, Lohi H, Munteanu I, et al. A previously unidentified MECP2 open reading frame defines a new protein isoform relevant to Rett syndrome. *Nat Genet* 2004;36:339–341.
9. Erlandson A, Samuelsson L, Hagberg B, et al. Multiplex ligation-dependent probe amplification (MLPA) detects large deletions in the MECP2 gene of Swedish Rett syndrome patients. *Genet Test* 2003;7:329–332.
10. Laccone F, Junemann I, Whatley S, et al. Large deletions of the MECP2 gene detected by gene dosage analysis in patients with Rett syndrome. *Hum Mutat* 2004;23:234–244.
11. Meins M, Lehmann J, Gerresheim F, et al. Submicroscopic duplication in Xq28 causes increased expression of the MECP2 gene in a boy with severe mental retardation and features of Rett syndrome. *J Med Genet* 2005;42:e12.
12. Van Esch H, Bauters M, Ignatius J, et al. Duplication of the MECP2 region is a frequent cause of severe mental retardation and progressive neurological symptoms in males. *Am J Hum Genet* 2005;77:442–453.
13. Friez MJ, Jones JR, Clarkson K, et al. Recurrent infections, hypotonia, and mental retardation caused by duplication of MECP2 and adjacent region in Xq28. *Pediatrics* 2006;118:e1687–e1695.
14. Neul JL, Fang P, Barrish J, et al. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. *Neurology* 2008;70:1313–1321.
15. Percy AK, Lane JB, Childers J, et al. Rett syndrome: North American database. *J Child Neurol* 2007;22:1338–1341.
16. Bebbington A, Anderson A, Ravine D, et al. Investigating genotype-phenotype relationships in Rett syndrome using an international data set. *Neurology* 2008;70:868–875.

Glut1 Deficiency: Inheritance Pattern Determined by Haploinsufficiency

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Two families manifesting Glut1 deficiency syndrome (DS) as an autosomal recessive trait are described. In 1

family, a severely affected boy inherited a mutated allele from his asymptomatic heterozygous mother. A de novo mutation developed in the paternal allele, producing compound heterozygosity. In another family, 2 mildly affected sisters inherited mutations from their asymptomatic heterozygous consanguineous parents. Red blood cell glucose uptake residual activity, a surrogate of haploinsufficiency, correlated with the clinical severity. These cases demonstrate that Glut1 DS may present as an autosomal recessive trait. The clinical pattern of inheritance is determined by the relative pathogenicity of the mutation and the resulting degree of haploinsufficiency.

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Glut1 deficiency syndrome (Glut1 DS) results from impaired glucose transport into the brain. Facilitated glucose transport is mediated by Glut1, a transmembrane protein encoded by the *GLUT1* gene.¹ The hallmark of Glut1 DS is low cerebrospinal fluid (CSF) glucose.² The classic presentation includes epilepsy, developmental delay, acquired microcephaly, spasticity, and ataxia. The expanded phenotype includes paroxysmal movement disorders^{2,3} and atypical childhood absence epilepsy.⁴

Glut1 DS often presents as a sporadic disease, with de novo mutations producing haploinsufficiency and conferring symptomatic heterozygosity.⁵ An autosomal dominant pattern of inheritance has been the Mendelian rule.^{6–8} In a haploinsufficient knockout mouse model for Glut1 DS, homozygosity confers embryonic lethality, and heterozygous mice show phenotypic similarities to the human condition.^{9,10}

We report 2 families in which the probands have 2 mutated alleles. The parents are clinically asymptomatic heterozygous carriers. The inheritance pattern is consistent with an autosomal recessive trait.

Subjects and Methods

Columbia Neurological Score

The patients were assessed for phenotypic severity using the Columbia Neurological Score (CNS), a semiquantitative tool to summarize the clinical evaluation.¹¹ The instrument rates physical and neurologic findings as normal or abnormal. The

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