ELECTRONIC LETTER

Patients with the R133C mutation: is their phenotype different from patients with Rett syndrome with other mutations?

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Rett syndrome is an X linked dominant neurodevelopmental disorder with an incidence of 1:10 000 females in Australia.¹ It is characterised by apparently normal development between 6 and 18 months, followed by a period of regression with loss of purposeful hand use, deceleration of head growth, and onset of repetitive, stereotypic hand movements.² Affected people also manifest gait ataxia and apraxia, autistic features, epileptic seizures, respiratory dysfunction, autonomic dysfunction, and decreased somatic growth.^{2,3} In recent years it has become apparent that the phenotypic range of this disorder is much wider than previously thought. Some patients may have a milder phenotype and retain the ability to walk or speak and others have an earlier onset and more severe features. People who have some but not all of the necessary criteria have been categorised as atypical⁴ or as one of six variant forms.⁵

Rett syndrome has now been shown to be associated with mutations in the methyl-CpG-binding protein 2 (MeCP2).⁶ For many genetic disorders, the next stage in research after the identification of the gene involves describing the relation between genotype and phenotype, and the phenotypic diversity produced by different mutations in the same gene. Some research has found that people with missense *MECP2* mutations may have a milder phenotype than those with truncating mutations.^{7,8} Weaving *et al*⁹ found that age at onset of hand stereotypies was later and speech and height (but not head growth) were slightly more normal in those with missense mutations whereas Nielsen *et al*¹⁰ found no difference in severity between these mutation types. In the study of Amir *et al*¹¹ breathing abnormalities were found to be more common with truncating mutations and scoliosis more common with missense mutations. Hoffbuhr *et al*¹² concluded that patients with missense mutations in the methyl binding domain (MBD) and mutations truncating the entire transcription repression domain (TRD) were more severely affected than those with missense and nonsense mutations in the TRD and C terminal segment. Similarly, in another study, a milder phenotype was associated with late compared with early truncating mutations.¹³ In a recent publication, Huppke *et al*¹⁴ considered those mutations in the nuclear localisation signal (NLS) as a separate category from the other truncating mutations. Overall, they found that cases with mutations leading to a partial or complete truncation of the NLS were more severe than those with mutations downstream of the TRD. Although some phenotypic associations with different classes of mutations within *MECP2* are emerging, the reports are hampered by relatively small sample size. In one recent review, it was actually suggested that a simple relation between clinical severity and type of mutation may not even exist.¹⁵

The location of *MECP2* on the X chromosome makes it very likely that altered patterns of X inactivation also contribute to the phenotypic variation as seen in heterozygous females from the same family.¹⁶ The closeness of the correlation between

Key points

- Rett syndrome is now known to be caused by mutations in the *MECP2* gene.
- Over 200 pathogenic mutations have been identified with eight common ones accounting for two thirds of cases.
- Grouping of mutations is not providing consistent genotype-phenotype relations across studies and we suggest there is a need to examine individual mutations.
- We ask the question for clinicians: "Is the phenotype of a girl with an R133C mutation likely to differ from what one would normally expect in Rett syndrome?"
- We found that the phenotype of a patient with an R133C mutation is milder overall with better ambulation and hand use and a greater likelihood of being able to use speech.
- It is important for clinicians to have this information when such patients are diagnosed.

skewed X inactivation patterns and the phenotype seen remains uncertain. In the two most comprehensive reported studies, Hoffbuhr *et al*¹² found skewing (defined as >85% of one X allele active) in 6/39 (15%) cases whereas Weaving *et al*⁹ with a more liberal definition (>75% of one X allele active) found it in 31/72 (43%).

Functional analysis of specific mutations associated with Rett syndrome is now also being undertaken. Similar to some other missense mutations in the MBD, the R133C mutation has been shown to abrogate methylation specific binding to the DNA template.¹⁷ However, in a later study Kudo *et al*¹⁸ found the R133C mutant to be functionally almost equivalent to the wild type protein. If this were the case, it could be hypothesised that people with the R133C mutation may have a milder phenotype.

The purpose of this study was to examine whether the clinical phenotype of patients with the R133C mutation is different from the clinical phenotype in those with other pathogenic mutations. Phenotype has been defined by scales that have already been used to describe the clinical variation in the Australian cohort.¹⁹ Although included in the group of eight

Abbreviations: DHPLC, denaturing high performance liquid chromatography; EDTA, ethylene diamine tetraacetic acid; FQ2000, Australian follow up 2000 study instrument; MBD, methyl binding domain; MeCP2, methyl-CpG-binding protein 2; NLS, nuclear localisation signal; PCR, polymerase chain reaction; RetfBASE, IRSA *MECP2* gene variation database; TRD, transcription repression domain; WeeFIM, functional independence measure for children

Table 1 Study demographics

	All R133C cases	Australia	Japan	United Kingdom	Other mutations
No	24	11	4	9	98
Age (years)					
Mean	14.8	14.6	10.4	17.0	13.9
SD	7.0	8.2	1.2	6.3	6.2
Min	4.9	4.9	9.6	5.5	2.0
Max	31.1	31.1	12.2	28.0	24.6
p value, <i>t</i> test*	0.5444	0.7477	0.2698	0.1575	
X inactivation					
Random	13	6	2	5	41
Skewed	2	2	0	0	29
Not tested	9	3	2	4	28
p value, <i>t</i> test†	0.0217	0.2269			
Classification‡					
Mild (22%)		3	3		19
Classical (55%)		7	0		55
Early onset (23%)		1	1		24

*Comparing mean to other mutation group mean; †comparing mean of $\log(P/(1-P))$, where P is the percentage of the less commonly expressed allele; ‡patients (9) from the United Kingdom not classified.

common mutations which account for almost two thirds of pathogenic *MECP2* mutations;²⁰ the R133C mutation currently represents only 4.1% of all *MECP2* variations reported in the IRSA *MEC2* gene variation database (RettBASE).²¹ To enhance the power of the analysis the R133C case group of 11 Australians was expanded by the addition of four Japanese and nine United Kingdom patients with this mutation.

MATERIALS AND METHODS

The Australian Rett Syndrome Database is an ongoing national registry of patients diagnosed with Rett syndrome born in 1976 and subsequently.²² At the end of 2001, 227 verified cases had been reported to the registry. Fourteen (6%) of these had died. Mutation screening had been carried out on 175 (77%) of these patients as well as on 19 Australian adult patients with a clinical diagnosis of Rett syndrome. A pathogenic mutation was identified in 121/175 (69%) of registry patients and 15/19 (79%) adult patients. Australian patients with an R133C mutation represented 9/121 (7%) registry cases and 2/15 (13%) adult patients with a pathogenic mutation. X inactivation studies have now been completed on 145 registry patients, including 8/9 with R133C mutations. X inactivation data were also available for 2/4 Japanese R133C patients and 5/9 United Kingdom R133C patients (table 1).

The Australian follow up 2000 study instrument (FQ2000) consists of 15 sections, one of which is a questionnaire version of the functional independence measure for children (the WeeFIM).²³ It also contains items which allowed us to provide Rett syndrome severity scores for three different scales which we have termed the Kerr, Percy, and Pineda scales.¹⁹ The Kerr scale was originally published to provide guidelines for describing the clinical features of patients with *MECP2* mutations.²⁴ Parents of seven of the nine registry patients with the R133C mutation completed the FQ2000 from the follow up study, as did the parent of one adult patient with the R133C mutation. For the other three Australian patients with the R133C mutation, we used data from the original family and clinicians' questionnaires and a telephone interview with one parent to obtain the necessary data items. Parents of all 98 people on the database with a positive mutation in the comparison group completed the FQ2000.

For the Japanese patients, a brief protocol was developed to capture the same information as provided by the FQ2000 questionnaire. The WeeFIM section was translated into Japanese and administered by telephone in one case and by interview with parents in three cases. For the patients in the United

Kingdom, clinical data in the format published in Cheadle *et al*⁷ were sought using a clinical data sheet that recorded key diagnostic points.²⁴

Chi-squared tests were performed on categorical variables and *t* tests were used to compare means of the continuous variables with the SAS package.²⁵ Growth charts for Japanese females were used to assess Z scores and centile ranges for the four Japanese patients. Centre for Disease Control growth charts²⁶ were used for the rest of the cohort.

Mutation analysis

DNA was extracted from blood samples collected in ethylene diamine tetra-acetic acid (EDTA) anticoagulant. Using a variety of template primers,⁷ the *MECP2* coding region was amplified by polymerase chain reaction (PCR) and then screened for mutations by direct sequencing (bidirectional).⁷ Screening was also performed in some cases by denaturing high performance liquid chromatography (DHPLC) followed up by sequence analysis of fragments shown to have an altered elution profile by DHPLC.⁹

Inactivation of the X chromosome

Inactivation of the X chromosome was measured by examining the methylation of each allele of the androgen receptor locus, after the method of Pegoraro *et al*.²⁷ Microsatellites were analysed using Genescan (Automated DNA Analysis Facility, University of New South Wales, Sydney, Australia). For scoring the X inactivation pattern, we calculated the percentage of the smaller allele present. This was converted to a logistic transformation²⁸ and values for R133C and comparison cases were compared with *t* tests.²⁵ Skewing was defined as greater than 75% of one X allele active.

RESULTS

For this study, the case population consisted of 11 Australian, four Japanese, and nine United Kingdom patients with Rett syndrome, who have been identified as having an R133C mutation. Four clinical scales, the WeeFIM, Percy, Kerr, and Pineda scales, have been used to compare this case group with the group within the Australian follow up 2000 study of 98 patients considered to have a pathogenic mutation other than R133C.

Table 1 shows the age distribution, country of ascertainment, and clinical classifications for case and comparison groups. There were six major groups of genotype based on type and domain which covered 93% of the 98 comparison case

Table 2 Individual clinical data items (categorical variables): R133C cases and comparison group

	R133C cases (24)			Other mutations (98)	
	p value	Percentage in category	Missing	Percentage in category	Missing
Feeding	<0.0001		4		2
Uses spoon		35		0	
Finger feeds		20		22	
No attempt		45		78	
Speech	<0.0001		1		5
More than single words		9		2	
Single words		48		2	
More than no vocalisation but no single words		17		66	
Lost speech		22		30	
Never acquired		4		0	
Gross motor function	<0.0001		0		5
Walks normally		54		12	
Walking impaired		38		27	
Previously or has never walked		8		61	
Age at losing social interaction	0.0011		10*		6
>18 months		86		39	
6–18 months		14		61	
Sleep disturbance	0.0013		9†		1
No disturbance reported		40		8	
Disturbance either past or present		27		22	
Disturbance both past and present		33		70	
Hand use	0.0017		3		1
Acquired and conserved		29		7	
Lost purposefulness 2–6 years or conserved manipulation		29		21	
Lost purposefulness <2 years or conserved grasping		33		18	
Acquired and lost		10		53	
Never acquired		0		2	
Respiratory	0.0075		2		6
No dysfunction		45		18	
Hyperventilation and/or apnoea		55		82	
Disturbed awake breathing rhythm	0.0102		5		6
Never		53		20	
Rare to daily		21		39	
Daily to constantly		26		41	
Frequency of hand stereotypies	0.0292		5		5
Never		5		1	
Rare to dominating		21		5	
Dominating to constantly		74		94	
Voluntary hand use	0.0307		4		1
Normal, feeding independently		20		7	
Some hand use, feeding with help		55		38	
None		25		55	
Scoliosis	0.0332		7‡		1
No scoliosis		65		38	
Scoliosis		35		36	
Scoliosis operated		0		26	
Ambulation	0.0233		9†		2
First walked <18 months and still walking		53		35	
First walked <18 months then lost ability, or first walked 18–30 months and still walking		20		16	
First walked 18–30 months and still walking		20		5	
First walked 18–30 months then lost ability		7		4	
First walked >30 months and still walking		0		40	
Epilepsy	0.0608		5		12
Never		21		27	
Previous seizures, or present and controlled with medication		74		50	
Uncontrolled		0		22	
Early epilepsy <12 months		5		1	
Present weight	0.0877		7§		8
Z score >=–1		59		32	
–2 < Z score <–1		6		20	
Z score <=–2		35		48	
Early developmental progress	0.0998		1		5
Normal progress		61		39	
Suboptimal progress		39		54	
No or virtually no progress		0		8	
Present HC	0.1567		5		6
Above 10th centile		53		35	
3rd–10th centile		26		21	
Below 3rd centile		21		45	
Present height	0.1980		8§		8
Z score >=–1		25		31	
–2 < Z score <–1		31		13	
Z score <=–2		44		56	

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Table 2 continued

	R133C cases (24)			Other mutations (98)	
	p value	Percentage in category	Missing	Percentage in category	Missing
Age at sitting alone	0.7770		11¶		7
Acquired <8 months		77		69	
Acquired 8–16 months		23		29	
Never acquired		0		2	

*Includes eight patients from the United Kingdom, one from Japan, and one from Australia; †includes all nine patients from the United Kingdom; ‡includes six patients from the United Kingdom; §includes seven patients from the United Kingdom; ¶includes all nine patients from the United Kingdom and two from Australia.

mutations. These were missense MBD (24.5%), nonsense TRD-NLS (21.4%) frameshifts in the C terminal (13.3%), missense TRD (12.2%), and nonsense mutations positioned between the MBD and the TRD (9.2%). The most frequent mutations found in the 98 comparison cases were T158M (n=14), R168X (n=11), R270X (n=11), R294X (n=9), R255X (n=8), and R306C (n=7).

The proportion of cases in each category for the R133C case group (n=24) for selected categorical items used in the adapted Kerr, Percy, and Pineda scales are contrasted with the comparison group (n=98) in table 2. Results for continuous variables and the four severity scales are shown in table 3.

Patients were significantly less likely to be in the most severe category for most of the individual clinical data items:

- Hand use (voluntary and finger feeding)
- Speech
- Gross motor function and ambulation
- Age at losing social interaction
- Sleep disturbance
- Respiratory dysfunction
- Awake breathing rhythm
- Age at onset and frequency of hand stereotypies
- Scoliosis.

Also, all four severity scales were significantly different between the patients with the R133C mutation and the comparison group.

X inactivation status was available for 15 R133C cases and 70 of the comparison group. In the Australian cases and comparison group, skewing was found in 31/78 (40%) cases. Skewing was less common in the R133C cases than the comparison group (p=0.0217) with the mean allele percentages for the less commonly expressed allele being 35.8% for cases and 27.1% for the comparison group (logistic transformations –0.27 and –0.50 respectively). Kerr, Percy, and Pineda scales

were uniformly but not significantly higher (24.5, 24.0, and 14.2) in the two cases with skewed than in the eight with random X inactivation (15.9, 17.6, and 11.1; p=0.09, 0.37, 0.41). Similarly, the mean WeeFIM score was lower (23.0) in the two with skewed, compared with 53.4 in the seven with random X inactivation (p=0.23).

DISCUSSION

This is the first report among cases of Rett syndrome that shows and measures the extent of the milder phenotype associated with the R133C mutation by contrast with cases that have mutations elsewhere in *MECP2*. These data show that patients harbouring the R133C mutation have better function overall. These girls and young women are more likely to have learned to walk and then remain ambulatory, to have better speech (single words or better), and to be able to use a spoon or finger feed. Among patients with the R133C mutation, hand stereotypies were less dominating and age of onset was later. Scoliosis, breathing, and sleep disturbances were less common.

Of interest is the fact that we have found no evidence to suggest that the mild phenotype in R133C cases is related to non-random X inactivation patterns. X inactivation was in fact more likely to be random in this group than in the comparison cases. This contrasts to the findings of Hoffbuhr *et al*¹² who found a linear relation between X inactivation ratios and clinical severity score for a small group of cases with missense MBD and nonsense mutations in the interdomain region. However, their complete study group of 73 cases with mutations only contained two cases with the R133C mutation and it is not known whether these were included in the group of 35 patients who had informative X inactivation results.

Although the quantitative data from the individual items and the composite scoring systems showed significantly milder disease overall, some R133C cases can be identified with phenotypes and severity scales in the range compatible with classical Rett syndrome. However, although equivalent

Table 3 Individual clinical data items (continuous variables) and severity scales: Australian R133C cases and comparison group

	R133C cases (11)				Other mutations (98)		
	Mean	SD	p value	Missing	Mean	SD	Missing
Age at onset of hand stereotypies (months)	41.1	14.2	0.0149	2	28.3	14.6	22
Age at onset of epilepsy (years)	7.0	8.0	0.0825	3	4.7	2.4	34
Latest weight Z score	–1.98	2.6	0.4800	0	–2.7	3.4	8
Latest height Z score	–2.59	1.2	0.5999	1	–2.3	1.9	8
Age at sitting alone (months)	7.7	1.8	0.8088	3	8.5	9.9	8
Age at onset of regression (months)	32.1	25.3	0.9763	2	32.3	22.7	5
Pineda scale (max=31)	12.1	3.7	0.0028	0	16.2	4.3	0
WeeFIM score (max=126)	37.9	17.9	0.0285	2	28.4	11.6	2
Kerr scale (max=37)	18.8	4.6	0.0261	0	22.0	4.5	0
Percy scale (max=47)	20.7	6.4	0.0455	0	24.8	6.5	0

Table 4 References to the *MECP2* R133C mutation in Rett syndrome in published reports

Study	No of patients	Country	Case ID	Phenotype data provided
Amano <i>et al</i> ³³	2	Japan	Seg014	Genotype data only provided
Uchino <i>et al</i> ³¹			Seg024	Uchino adds "had words" only
Amir <i>et al</i> ⁶	2	United States of America		Analysed as missense v truncating
Amir <i>et al</i> ³⁴				
Amir <i>et al</i> ¹¹				
Buyse <i>et al</i> ³⁵				
Sung <i>et al</i> ³⁶				
Wan <i>et al</i> ¹⁶				
Auranen <i>et al</i> ³⁷	3	Finland		Overall milder clinical phenotype; all learnt to walk and developed normally <10 months
			R24	Born 1995; hands: uses purposefully
			R26	No epilepsy; no scoliosis
			R47	Born 1979; walking: until 9–10 years
Chae <i>et al</i> ³⁸	1	Korea	17	Born 1975; obese
				Compared missense and nonsense severity scores
				Age: 9 years 3 months
				Hands: grasps briefly
				Speech: a few words
				Walking: walks alone
				Epilepsy; no abnormal respiration
				Hands: uses spoon/finger feeds/holds a cup or bottle
Cheadle <i>et al</i> [*]	4 includes 2 sisters	United Kingdom	6	Speech: few words, possibly with meaning
			10	Walking: independently
				Hands: grasps or holds briefly
				Speech: few words, possibly with meaning
			81	Walking: independently
				Hands and speech: not available
				Walking: independently
			84	Non-classical
				Hands: uses spoon/finger feeds/holds a cup or bottle
				Speech: understands and says some words
				Walking: independently
Erlandson <i>et al</i> ³⁹	1	Sweden		No phenotype data; different case than that reported by Xiang <i>et al</i> ⁴⁰
Hoffbuhr <i>et al</i> ¹⁵	1	United States of America	R115a	Hands: limited preserved hand use
				Speech: preserved
				Walking: ambulatory
Huppke <i>et al</i> ¹³	1	Germany	26	Decelerated head circumference; seizures
				No loss of ability to sit, walk, or speak.
				Head circumference >3rd centile
Laccone <i>et al</i> ⁴¹	6			No phenotype data
Trappe <i>et al</i> ⁴²	3			No phenotype data; mutations 2 paternal, 1 maternal origin
Inui <i>et al</i> ²⁰	2	Japan	3	14 years, no gait apraxia
			7	8 years, no head growth deceleration; no severely impaired language; no severe psychomotor retardation
Milunsky <i>et al</i> ⁴³	5	United States of America		Some cases without microcephaly
Nielsen <i>et al</i> ¹⁰	1	Denmark	6	Born 1972
				Hand: can hold glass, uses spoon or fork
				Gait: walks independently
				Eye gaze: intense, uses eye pointing
Nielsen <i>et al</i> ⁴⁴	1	Denmark	2	Born 1970; preserved speech variant
				Hands: uses hands for eating and drinking
				Speech: lost speech at 5 years, regained at 6 years at poorer level (short sentences)
				Walking: relatively effortlessly
				Head circumference normal; no scoliosis or kyphosis
				No phenotype data provided
Vacca <i>et al</i> ^{45 46}	3	Italy/United Kingdom	N4	
			N19	All United Kingdom cases
			N24	
Xiang <i>et al</i> ⁴⁰	1	Sweden	41	No phenotype data provided
Yamada <i>et al</i> ⁴⁷	2	Japan	R02	Diagnosed at 11 years
				Speech: says a few words, possibly with meaning
				Walking: since 17 months
				Epilepsy; no head growth deceleration
			R17	Diagnosed at 17 years
				Speech: none
				Walking: not walking
				Epilepsy
Yamashita <i>et al</i> ^{29†}	2	Japan	1	9 years, onset hand stereotypies at 6 years
				Hands: eats with spoon

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Table 4 continued

Study	No. of patients	Country	Case ID	Phenotype data provided
Kondo and Yamagata ^{48†}	+5		3	Speech: short sentences Walking: at 7 years could run and inline skate; normal growth 9 years, preserved speech variant, onset hand stereotypies at 5 years Hands: hand function relatively preserved Speech: speaks 3–4 words Walking: unsupported onset of epilepsy at 7 years, no microcephaly
Zappella <i>et al</i> ⁸²	2	Italy	368.CR 307.FR	10 years, no scoliosis; first words >24 months 30 years, no seizures; first words >24 months; >2 word sentences

*Three cases are included in the United Kingdom cases for the current study; †Japanese cases included in the current study.

information on their representativeness is not available for the cases from either the Japanese or the United Kingdom, we know that the cases from Australia have been sourced from a national population database and are thus representative of patients with the R133C mutation in this country. The fact that such a sound epidemiological framework has underpinned this particular study provides us with the confidence to generalise the findings. Within R133C cases we do not know what factors predict a milder or more severe phenotype but we have found no evidence to suggest that skewed X inactivation is protective (but rather noted a tendency to the reverse). As noted by Hoffbuhr *et al*¹² relative to their six cases which showed preferential use of one X chromosome, patients with skewing seem to be either more mildly or more severely affected than other patients with the same type of mutation. Like Hoffbuhr *et al*,¹² in this study we were unable to determine whether it was the normal or mutated X chromosome that was preferentially active in those patients with skewing. If it were the mutated chromosome this could account for the direction of our findings.

Until now, reports of patients with the R133C mutation have involved only a description of affected patients who also have given an impression of milder disease (table 4). The R133C mutation has previously been documented in girls with preserved speech^{29–32} and in a family with two mildly affected sisters and a clinically unaffected mother.⁷ Based on a case sample with a total of only 10 patients with preserved speech (two of whom had an R133C mutation) and with no comparison group, Zappella *et al*⁸² have commented that the presence of this mutation is one factor likely to contribute to a milder phenotype. Ability to confirm this association statistically in our study depended upon the existence of the Rett syndrome epidemiological database in which most patients have now been genetically screened. However, because Rett syndrome is a rare disorder and because fewer than 1/20 of these patients may have the R133C mutation, it takes this optimal level of ascertainment of juvenile Rett syndrome in the Australian total population of almost 19.5 million people to generate nine such affected childhood cases. As well as adequate sample size, which is clearly facilitated by national population based data collections as well as international collaborations, the two other important ingredients necessary for success are comprehensive mutation analysis and systematic collection of relevant clinical data.

The systems for classification of phenotypes which have been developed so far have partly resulted from individual researchers taking advantage of the information they had already collected on various clinical items and using these to develop a score.^{7,8,11,14} The scores for two (Percy and Kerr) of the scales used to describe the phenotype in our Australian population database¹⁹ increased with age. This did not occur with the Pineda scale (derived from the study of Monros *et al*⁸) which was based more on developmental data than on current clinical features. Because the milder phenotype associated with the R133C mutation is so marked, confounding by age

was not likely to be a problem in this study. However, this may be an issue in future studies.

Although around 200 different pathogenic mutations have now been identified in the *MECP2* gene,²¹ it is thought that eight common mutations represent about two thirds of the total number of pathogenic mutations.²⁰ Among these eight recurrent mutations, R133C is one of three missense mutations in the MBD along with R106W and T158M, the commonest of all mutations. Even in the more complex classification systems,¹² these two (R133C and T158M) would be grouped together as missense MBD mutations but in reality may have very different phenotypes. Despite including what we have now shown to be a mild mutation, missense MBD mutations were estimated to be the second most clinically severe group in the analysis of Hoffbuhr *et al*.¹² Therefore, it is now apparent that these common mutations must be assessed individually rather than being treated as part of a larger group. The R133C mutation may well be at one end of the clinical range of Rett syndrome phenotypes associated with *MECP2* mutations. As this is clinically useful information it is apparent that each of the other recurrent mutations also requires more detailed evaluation.

In recommending that future genotype-phenotype analyses should focus on individual mutations rather than groupings, it is important to consider the reasons for undertaking such studies of genotype-phenotype. The primary reason is to gather information which will allow clinicians to assess the likely clinical course for children in whom the diagnosis of Rett syndrome has been confirmed by molecular testing. In the past, the need for adherence to clinical criteria to make the diagnosis has constrained clinicians in their diagnostic practice and perhaps resulted in missing those atypical patients, who have symptoms which are either milder or more severe than classical Rett syndrome. Our experience with the R133C mutation further suggests that the phenotypic boundaries of Rett syndrome extend well beyond the original Trevathan criteria.³ It is important not only that a diagnosis of Rett syndrome is confirmed in this group of children but also that clinicians are aware of their generally milder phenotype when counselling families about future prognosis.

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