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Specific Mutations in *Methyl-CpG-Binding Protein 2* Confer Different Severity in Rett Syndrome

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Abstract

Objective—To determine if a relationship exists between the clinical features of Rett Syndrome, an X-linked dominant neurodevelopmental disorder, and specific mutations in *MECP2*.

Method—Cross-sectional study of two hundred and forty-five girls and women with typical Rett Syndrome seen between 1990 and 2004 in tertiary academic out-patient specialty clinics and who had complete *MECP2* mutation analysis. A structured clinical evaluation was completed for each participant. The results were grouped by *MECP2* mutation and compared.

Results—Participants with the R133C mutation are less severely affected than those with R168X or large DNA deletions ($p<0.05$). Likewise, individuals with the R168X mutation are more severely affected than those with R294X and late carboxy-terminal truncating mutations ($p<0.05$). Clinical differences are notable in ambulation, hand use, and language, ($p<0.004$) three cardinal features of Rett Syndrome. Individuals with R168X are less likely to walk ($p=0.008$), retain hand use ($p=0.002$), or use words ($p=0.001$). In contrast, those with carboxy-terminal truncations are more likely to walk ($p=0.007$) and use words ($p<0.001$). The R306C mutation, previously found to confer milder features, adversely affects only one clinical feature, language ($p<0.05$).

Conclusions—Specific mutations in *MECP2* confer different severity. These results allow the design of therapies targeted towards the amelioration of expected problems. Furthermore, the distinct effects of *MECP2* mutations on clinical severity must be considered in clinical intervention trials.

Keywords

Rett Syndrome 318; Genetics 91; Developmental Disorders 228; Mental retardation 229

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Introduction

Rett Syndrome (RTT) is an X-linked dominant neurodevelopmental disorder which occurs in 1.09/10,000 females[1] and is characterized by regression of language and hand use[2]. Hand stereotypies are characteristic, occurring during wakefulness and interfering with purposeful hand use. Ambulation is often disturbed. Furthermore, a number of other neurological problems including tremor, chorea, dystonia, and epilepsy are common. Affected individuals have decreased somatic and brain growth and autonomic abnormalities such as breathing irregularities and cold, blue extremities[3]. Typical RTT is diagnosed based on a set of clinical criteria (E-Table 1)[4]. Atypical RTT, which can be milder or more severe than typical RTT, is diagnosed when some, but not all, of the typical RTT clinical criteria are present[4].

Mutations in the gene encoding *Methyl-CpG-binding protein 2* (*MECP2*) cause the majority of individuals with typical RTT[5]. Although over 200 unique mutations in *MECP2* cause RTT (*Rettbase;* http://mecp2.chw.edu.au/), eight common mutations (R106W, R133C, T158M, R168X, R255X, R270X, R294X, R306C) account for more than 60% of typical RTT individuals. In addition, a number of small insertions/deletions in the 3′ end of the gene lead to carboxy-terminal truncations (C-terminal truncations). The molecular similarity of these mutations justifies considering them as a group, accounting for 5-10% of typical RTT (*Rettbase;* http://mecp2.chw.edu.au/). Mutations in *MECP2* have also been discovered in atypical RTT[6] and in other neurodevelopmental disorders, such as autism[7, 8, 9, 10], Angelman-like syndrome[11, 12], and non-specific mental retardation[13, 14].

Although the features of typical RTT are distinctive, affected individuals display clinical variability. For example, some individuals with typical RTT are able to walk unassisted whereas others are completely non-ambulatory. One proposed explanation for this variability is non-random X-chromosome inactivation (XCI). XCI is the inactivation of one of the two X-chromosome in every female cell which usually results in a random distribution of active X-chromosomes in the adult. Although variation in XCI can explain some of the variance in severity of individuals with RTT (approximately 20%)[15], it does not fully account for the range of clinical severity seen in typical RTT.

This begs the question: Do specific *MECP2* mutations result in particular clinical features in typical RTT? The fact that some mutations are more common in mild atypical RTT [6, 9, 16-21] supports the notion that specific *MECP2* mutations contribute significantly to the clinical variation in typical RTT. If these *MECP2* mutations are more frequently present in mild atypical RTT, they may also represent some of the less severe typical RTT individuals. Indeed, individuals with R133C are less severely affected[22]. Previous genotype/phenotype correlations lacked the discerning power to detect differences between specific mutations[23-30]. In this study, we present a large, cross-sectional cohort of strictly defined individuals with typical RTT [4] (E-Table 1). Common point mutations, large deletions, and C-terminal truncations are compared and distinct differences in clinical severity identified. The comparison of clinical features in RTT resulting from specific *MECP2* mutations provides information that will be useful in guiding therapeutic interventions, designing clinical intervention trials, and understanding the molecular nature of the MeCP2 protein.

Methods

Patients and clinical evaluation

The protocol and consent form were approved by the Institutional Review Boards of Baylor College of Medicine (BCM) and the University of Alabama - Birmingham (UAB). Parents or legal guardians of the participants gave informed consent. Participants were seen at either the Blue Bird Circle Rett Center at Texas Children's Hospital (TCH), BCM, or UAB Rett Center

between 1990 and 2004. Forty-five participants from UAB [31] and sixty-nine from TCH [32] were included in previous studies. A history and structured examination was performed on each girl by experienced examiners (DG, AP) to confirm the diagnosis using consensus criteria[4]. Disease severity was determined using a clinical rating scale [Clinical Severity Score (CSS); E-Table 2] which was developed specifically for RTT. The CSS is a composite score based on thirteen individual, ordinal categories measuring clinical features common in Rett syndrome. All scores range from 0 to 4 or 0 to 5 with 0 representing the least severe and 4 or 5 representing the most severe finding. A simplified scoring system was used to compress the ordinal category measures into a binary measurement with 0 representing "mild" or "retained function" and 1 representing "severe" or "lost/absent function". The clinical interpretation for the score 0 is given in E-Table 3. From this compressed clinical severity score, the percentage of individuals with mild/retained function can be generated for each clinical category. Individuals were included if they meet the consensus criteria for typical RTT (requires all the main criteria listed in E-Table 1 except deceleration in head growth), had complete testing for *MECP2* mutations (as outlined below, including testing for large DNA rearrangements), and had a complete CSS assessed. We excluded forty individuals who had complete *MECP2* mutation analysis and a complete CSS but did not meet the criteria for typical Rett syndrome but rather atypical RTT. The rationale for excluding individuals with atypical RTT is that they often represent the extreme ends of the phenotypic spectrum, both milder and more severe than typical RTT. The overall results were unchanged when these individuals were included in the analysis (data not shown).

MECP2 mutation analysis

Participants in this study had complete *MECP2* mutation analysis performed including sequencing exon 1 and evaluation for large DNA rearrangements by Southern blotting or by multiple ligation-dependent probe amplification (MLPA) analysis. *MECP2* mutation analysis was performed either by the Baylor DNA Diagnostic Laboratory[33, 34], or by the Greenwood Genetics Laboratory (http://www.ggc.org/diagnostics/molecular/rett_syndrome.htm). X-inactivation analysis was performed based on the protocol described previously[35].

Statistical analysis

Analyses were performed using SPSS v.12 (SPSS, Chicago IL) or SAS (SAS Institute, Cary, NC). *MECP2* mutation groups were compared on continuous variables (age, total CSS) using ANOVA with post-hoc tests conducted using Tukey's honestly significant difference test. For comparisons of the individual CSS categories, which are ordinal data, non-parametric statistics (Kruskal-Wallis test for K groups, Mann-Whitney U tests for pair-wise comparisons) were used. Proportional data was analyzed using Pearson χ^2 analysis (or where the expected number of cell counts was less than 5, a 2×K Fisher exact test), with a Bonferroni adjusted significance level for the overall difference ($p<0.004$) followed by a Tukey-style procedure (SAS Macro implementing procedure[36]) to detect pair-wise differences. Relative risk was calculated for those pair-wise differences that were significant ($p<0.05$).

Two approaches were used to minimize Type I errors. First, defined procedures to account for multiple comparisons were used. For example, parametric analyses were followed by defined post-hoc procedures. Similarly, post-hoc testing of proportional data was analyzed using Tukey-style multiple comparisons of proportions[36]. When no formal correction method exists, we adjusted the significance threshold using a Bonferroni correction. For example, when comparing *MECP2* mutation groups across the 13 clinical categories, the adjusted significance level is $p=0.5/13=0.004$.

Secondly, we minimized the number of statistical tests performed. After determining which pairs of mutation groups were different on total CSS and which clinical categories had overall

differences, pair-wise tests were performed only on those clinical categories that were different overall and only between those mutation pairs that were different in total CSS. Thus, the pair-wise comparisons were limited to 12 tests [(3 overall clinical category differences)×(4 pair-wise mutation differences in total CSS)] using Mann-Whitney U tests with a Bonferroni adjusted significance level (p=0.05/12=0.004).

Results

Description of participants

Two-hundred forty-five (245) girls and women seen at Texas Children's Hospital (TCH) or the University of Alabama-Birmingham (UAB) met the criteria for typical RTT[4](E-Table 1), had a Clinical Severity Score (CSS) (E-Table 2) assessed, and complete *MECP2* mutation analysis performed. Ninety-seven percent (236/245) have a mutation in *MECP2*. To determine if differences in clinical severity exist between different *MECP2* mutations, participants were placed into twelve mutation groups. Eight mutation groups consist of the most common point mutations (R106W, R133C, T158M, R168X, R255X, R270X, R294X, R306C). One individual with R133P and one with S134C were included in the R133C group and one participant with R306H was included in the R306C group. These common point mutations account for 67.4% of the total group. Two additional groups consist of clusters of molecularly similar mutations: large deletions (deletions of exon 3 and 4 or exon 4 alone, or complex insertions/rearrangements that disrupt the entire coding sequence), and mutations that cause late carboxy-terminal truncations (C-terminal truncations). The final two groups consist of all the remaining mutations (other mutations) and those participants with no *MECP2* mutations. The number, percentages, mean age at examination, and mean total CSS for each mutation group is shown in Table 1. We observe no difference in the mean age of examination between mutation groups [$F(11, 233)=1.664$, $p=0.083$]. Furthermore no correlation was found ($F(1;243)=0.126$, $p=0.723$) between CSS and age.

Overall clinical severity depends on *MECP2* mutation

The total CSS is different [$F(11, 233)=3.33$, $p=0.0003$] between the mutation groups (Figure 1, Table 1). Post-hoc analyses show pair-wise differences ($p<0.05$) between both R133C and R168X and between R133C and large rearrangements. Similarly, pair-wise differences exist between R168X and R133C, R294X, and C-terminal truncations for total CSS.

To determine if the effect of specific *MECP2* mutations is independent of XCI status, we performed XCI analysis on mutation groups that represent extreme ends of the clinical severity spectrum (R133C, R168X, R306C, and large deletions). We assessed the total CSS score for those participants that had random (less than 80:20%) XCI and found that R168X (n=12 with random XCI) has a higher total CSS (26.6) compared with R133C (n=7 with random XCI, CSS=18.1, p=0.011) or with R306C (n=8 with random XCI, CSS=20.1, p=0.014). Furthermore, R133C shows a trend towards being less severe than large deletions (n=8, CSS=26.4, p=0.053). These results indicate that, in general, the overall clinical severity conferred by specific *MECP2* mutations is independent of XCI status.

MECP2 mutations confer differences in ambulation, hand use, and language

To determine if specific *MECP2* mutations result in differences in any of the clinical categories that make up the overall CSS, we analyzed each of the thirteen categories. Analyses reveal differences ($p<0.004$) between the *MECP2* mutations in the following categories: ambulation, hand use, and language. Pair-wise analyses, performed only between those mutation pairs that were different on the overall CSS score, reveal that ambulation is different between R168X and C-terminal truncations, and between R168X and R294X (Table 2), and trends towards difference between R168X and R133C (p=0.005). Hand-use is different between R168X and

R133C, and between R294X and C-terminal truncations (Table 2). Hand use also trends towards difference between R133C and large rearrangements (p=0.0044). Language is different between R168X and R133C, and between R294X and C-terminal truncations (Table 2).

Retention of function depends on specific *MECP2* mutations

We assessed the percentage of individuals with a given mutation who retain meaningful skills or are only mildly affected. For example, in the ambulation category, a score of 2 or less indicates the ability to walk alone, whereas a score of 3 or higher indicates that the individual cannot walk unaided or is completely unable to walk (Table 2, E-Table 2). Similar divisions can be made for all the clinical categories in the CSS to reflect whether the individual is mildly affected (*i.e.* retains function or symptomatically mild) or severely affected (*i.e.* lacks function or symptomatically severe). E-Table 3 outlines the clinical definition for the mildly affected criteria for each category used to generate a compressed clinical severity scoring system. Using this simplified system, we determine the percentage of individuals with a specific *MECP2* mutation that retain function or is minimal impaired for each clinical category. Implementing this approach, we find that individuals with specific *MECP2* mutations differ in their ability to walk independently, use their hands, and use words ($p<0.004$). The percentages of individuals with retained function for these categories are shown in Table 3.

Pair-wise tests confirm that language is more retained in R133C compared with R168X, in R294X compared with R168X, in other mutations compared with R168X, in C-terminal truncations compared with R168X, in C-terminal truncations compared with R306C, and in C-terminal truncations compared with large deletions (all $p<0.05$). The absolute risk and relative risk for each of these comparisons is shown in Table 4. In summary, the milder mutations (R133C, R294X, other mutations, c-terminal truncations) confer between 6.0-20.5 fold increased relative risk of having some word use compared with the severe mutations (R168X, large deletions, R306C).

The ability to walk alone is more likely in individuals with C-terminal truncations compared to those with R168X and in R294X compared to those with R168X (Table 3). There is approximately a 3-fold increase in the relative risk that the individuals with mild (C-terminal truncations or R294X) mutations will be able to walk alone compared to those with R168X (Table 4). Individuals with R168X also have decreased use of hands compared with R133C, C-terminal truncations, and other mutations (Table 3). Mild mutations (R133C, C-terminal truncations, other mutations) confer between 2.1-2.4 fold increased relative risk of retained hand use compared to R168X (Table 4).

Using this simplified scoring system, an interesting observation concerning the correlation of retained function within specific mutations is apparent. Whereas some of the mutation groups have a low percentage of individuals with retained skills, such as R168X and large deletions, and other mutations have a high percentage of individuals with retained skills, such as R133C and C-terminal truncations, an interesting dissociation of the retained function exists in those with R306C (Figure 2). Although the R306C group tends to be less affected in total CSS (Figure 1) and a high percentage of these individuals can walk alone (67%) and have some hand use (52%), only a small percentage (10%) use words. This percentage is different ($p<0.05$, Table 3) from the percentage of those with retained language in the C-terminal truncations group (RR, 7.4; Table 4). This unexpected dissociation of the degree of clinical severity between these three categories suggests that the molecular nature of R306C is unique and disrupts distinct functions of the MeCP2 protein.

Although the differences in clinical severity are important in understanding the molecular nature of different *MECP2* mutations, assessing the likelihood of retaining function given the

presence of a specific mutation could provide meaningful clinical information to assist in prediction of clinical features. This information could help guide interventions, such as physical therapy, to ameliorate a specific symptom. With this goal, we compared among the five specific mutations (R133C, R168X, R294X, R306C, and large deletions) the percentage of individuals with a specific mutation that retained function to the percentage of individuals without that mutation that retain function. Using a significance cutoff of $p<0.01$, individuals with R168X are approximately one half as likely to be able to walk, one half as likely to be able to use hands, and one tenth as likely to be able to use words as those without R168X (Table 5). On the other hand, those with C-terminal truncations are almost twice as likely to walk alone and nearly three times as likely to use words as those without C-terminal truncations (Table 5). Individuals with R294X were also almost twice as likely to walk as those without R294X.

X chromosome inactivation and large deletions in *MECP2*

The bimodal distribution of large deletions in specific clinical categories (Table 2) is unexpected. Large deletions are expected to be more severe because deletions result in complete loss of the functioning protein. The fact that a large proportions of individuals with this mutation are less severely affected in hand use and ambulation is surprising. This may reflect non-random skewing of XCI due to selective pressure against complete loss of MeCP2 function. Sixteen of the 17 participants with large deletions had XCI status assessed and informative results were found in 14 of the 16 assessed. Eight have non-skewed XCI and 6 have skewed (more than 80:20) XCI. Within this group, XCI status does not confer a difference in the overall clinical severity or in any specific category. However, a high percentage (4/6, 67%) of the large deletion participants with skewed XCI are able to walk compared with only twenty-five percent (2/8) of the non-skewed XCI participants. This may indicate that the skewing of the XCI in these individuals favors the X-chromosome expressing the wild-type copy of *MECP2*.

Discussion

This study consists of a large series of individuals with typical RTT, formal clinical assessment, and complete *MECP2* mutation analysis. This allows comparisons of the severity between participants with common specific mutations. In general, specific *MECP2* mutations confer different clinical severity in typical RTT. Furthermore, specific mutations (R133C, R294X, C-terminal truncations) are less severe than other mutations (R168X, large deletions). These differences seem to be largely independent of XCI status. The difference in severity appears to result primarily from variation in three clinical features: ambulation, hand use, and language.

One particular mutation, R306C, has an unusual dissociation of the amount of preserved function. A large percentage of those with R306C are able to walk but very few are able to use words. In contrast, a previous study found that individuals with R306C have a milder phenotype[31] and specifically had better language skills. A significant difference between this study and the previous work is the inclusion of atypical RTT in that study and the exclusion of such atypical individuals in this study. The improved language skills in the R306C group in the prior study may be due primarily to inclusion of atypical RTT. This argues that the R306C mutation can either severely affect language thus causing typical RTT, or does not dramatically affect language and allows the expression of milder atypical RTT or non-RTT neurodevelopmental disorders. The clinical variability of this mutation suggests that it plays a major role in the function of the MeCP2 protein.

This study presents two features of clinical relevance. First, it provides the basis for clinical counseling. Although the study lacked the power to discern the severity of any specific common mutation, it does distinguish five mutations that represent the extremes of the severity spectrum. Furthermore, within these five mutations, predictions for the possible clinical outcomes can

be made. For example, this series shows that R168X confers an increased risk that the affected individual will not be able to walk, will not use words and will not have any retained hand use, whereas individuals with C-terminal truncations are much more likely to use words and walk alone. Such knowledge provides the framework for clinical counseling and assists in tailoring therapies towards the problems that result more frequently from particular *MECP2* mutations.

The second clinically relevant finding concerns the design of clinical trials for Rett syndrome. Because clear differences in clinical severity exist between mutations in Rett syndrome, any intervention trial must take this into account in study design. Without such planning, false negative and false positive results might occur due to a skewed distribution of mutations amongst the treatment groups. The simplest way to account for this is to design trials that compare individuals pre- and post-treatment.

A major point of this work is the need for a larger cohort to analyze for these genotype effects. Although this study has the largest clinical population of typical RTT published to date, the smallest mutation group (R106W) only represents 3.7% of the total population, necessitating a very large sample size to acquire an adequate number of individuals for this group. Because a number of genotype-phenotype comparisons have been performed in the past, it is possible to perform a meta-analysis on the published data to look for specific mutation effects. A challenge with such an analysis is the variation in the clinical rating systems used across studies. We propose that use of the simplified, compressed system presented here would allow an easy method to perform both a meta-analysis of previously published genotype-phenotype comparisons in RTT and the collation of existing international data sets for *de novo* analysis.

Beyond clinical relevance, this work highlights a *MECP2* allelic series which indicates that particular regions of the MeCP2 protein have unique genetic and protein interactions that determine dissociable functions of MeCP2. For example, the common missense mutations (R133C, R306C, T158M, and R106W) are dissimilar with respect to the overall clinical severity conferred by these mutations. Although R106W appears to disrupt interaction with methylated cytosines, the other mutations do not[37, 38]. This suggests that these mutations alter distinct functional and possible physical interactions. An alternative explanation is that these mutations may have different effects on the mRNA or protein stability, which could be tested experimentally. An interesting comparison is between R133C and R306C, both of which are relatively mild but have differential effects on language. Understanding the interactions disrupted by these specific mutations will help elucidate the molecular mechanisms involved in the acquisition and maintenance of important neurodevelopmental skills and will identify key proteins important for control of ambulation, language and hand use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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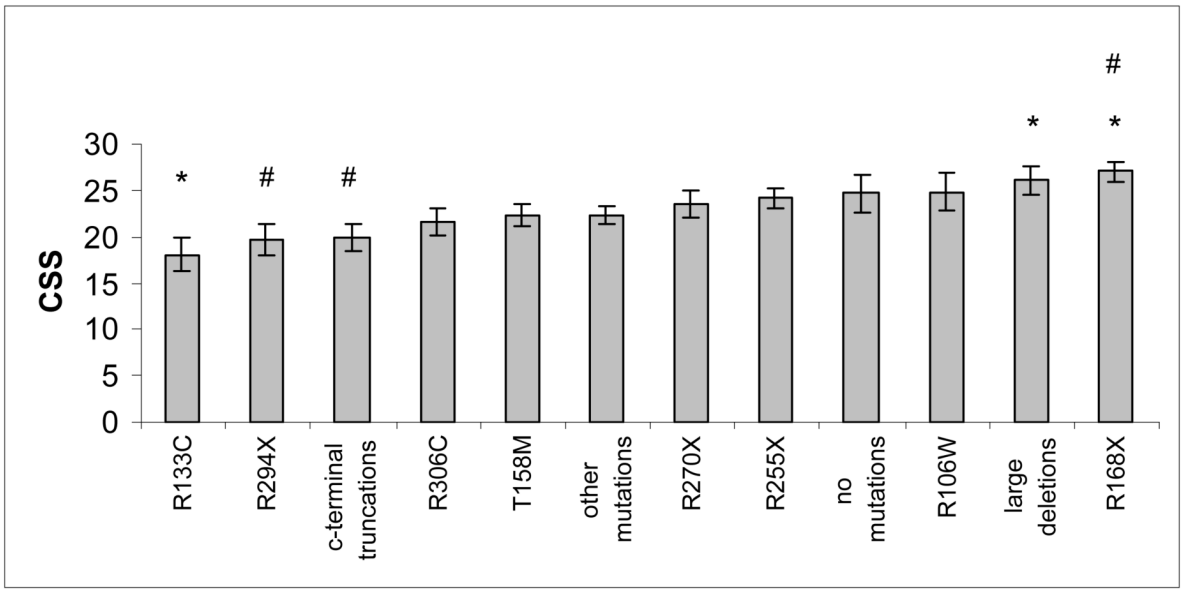

Figure 1. Clinical severity depends on specific *MECP2* mutations

There is an overall difference (p=0.0007) in the CSS between different mutations (listed along the x-axis). The asterisk (*) and the number sign (#) show significant (p<0.05) post-hoc pair-wise differences. Values represent mean ± SEM.

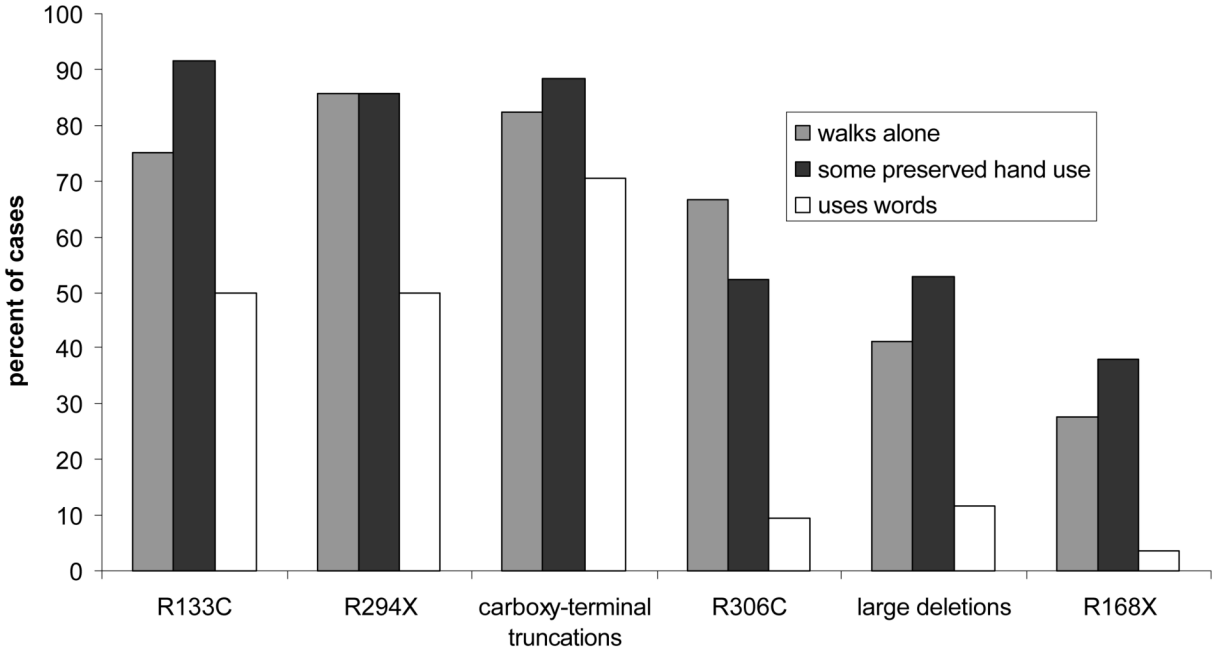

Figure 2. Dissociation of the clinical severity for individuals with R306C

The percentage of individuals with a given mutation (given along the x-axis) who are able to walk alone (grey), have some preserved hand use (black) or uses words (white) is shown along the y-axis.

Table 1

Characteristics of *MECP2* mutation groups.

Genotype	n	%	Mean age in mo. (95% CI)	Mean Total CSS (95% CI)
R106W	9	3.7	173 (111 --236)	24.8 (20.7 -- 28.8)
R133C	12	4.9	125 (71 -- 177)	18.1 (14.6 -- 21.6)
T158M	30	12.2	105 (71 -- 169)	22.3 (20.1 -- 24.6)
R168X	29	11.9	133 (98 -- 167)	27.0 (24.8 -- 29.3)
R255X	32	13.1	100 (67 -- 132)	24.2 (22.1 -- 26.4)
R270X	18	7.3	146 (102 -- 190)	23.6 (20.7 -- 26.4)
R294X	14	5.7	184 (134 -- 234)	19.7 (16.5 -- 23.0)
R306C	21	8.6	142 (101 -- 182)	21.6 (19.0 -- 24.3)
c-terminal truncations	17	6.9	128 (82 -- 173)	19.9 (17.0 -- 22.9)
large deletions	17	6.9	101 (56 -- 146)	26.1 (23.1 -- 29.0)
other mutations	37	15.1	105 (75 -- 136)	22.4 (20.4 -- 24.4)
no mutation	9	3.7	173 (111 -- 235)	24.7 (20.6 -- 28.7)
all	245	100	135 (121 -- 148)	22.9 (22.0 -- 23.7)

Table 2

Specific *MECP2* mutations cause differential severity in three clinical features: ambulation, hand use, and language

This table shows the percentage of individuals with a specific mutation and a clinical severity score on three of the clinical categories, hand use, language, and hand use. The clinical meaning for the numeric score is shown below the score. The asterisk (*), number sign (#) and carrot (^) indicates pair-wise differences between mutation groups on post-hoc testing ($p<0.004$). The total number of participants with a given mutation is shown in Table 1.

		Clinical	Severity	Subscale		
	mild			→	**severe**	
HAND USE	**0** conserved	**1** acquired on time, partially conserved	**2** acquired late, partially conserved	**3** acquired but lost	**4** never acquired	
R133C*	17%	75%	0%	8%	0%	
R294X#	0%	64%	21%	14%	0%	
C-term^	0%	76%	12%	12%	0%	
Large deletions	0%	41%	12%	41%	6%	
R168X*,# ^	0%	21%	17%	59%	3%	
LANGUAGE	**0** preserved	**1** short phrases	**2** single words	**3** babbling, vocalization	**4** screaming, or no utterances	
R133C*	0%	8%	42%	50%	0%	
R294X#	0%	7%	43%	43%	7%	
C-term^	0%	18%	53%	24%	6%	
Large deletions	0%	0%	12%	65%	24%	
168X*,#,,^	0%	0%	3%	86%	10%	
AMBULATION	**0** acquired <18mo	**1** 18mo≤acquired≤ 30 mo, walks alone	**2** acquired>30mo walks alone	**3** walks with help or acquired>50mo	**4** lost	**5** never acquired
R133C	58%	17%	0%	0%	25%	0%
R294X*	57%	21%	7%	7%	0%	7%
C-term#	35%	41%	6%	12%	6%	0%
Large deletions	35%	6%	0%	12%	18%	29%
R168X*,#	14%	10%	3%	24%	17%	31%

Table 3

Percentage of individuals with specific *MECP2* mutations that retain functional ability

All thirteen compressed subscales (E-Table 3) were compared and differences between all mutation groups were observed for ambulation, hand use, and language. Pair-wise testing between specific mutation groups revealed differences ($p<0.05$) shown by the asterisk (*) or the number sign (#).

genotype	n	walks alone (%)	uses hands (%)	uses words (%)
R106W	9	33	56	33
R133C	12	75	92*	50 *
T158M	30	60	50	27
R168X	29	28 *	38*	3 *
R255X	32	38	59	28
R270X	18	44	67	22
R294X	14	86 *	86	50 *
R306C	21	67	52	10 #
c-terminal truncations	17	82 *	88*	71 *#
large deletion	17	41	53	12 #
other mutations	37	43	78*	41 *
no mutation	9	33	78	33

Table 4

Absolute and relative risks of retained function between pairs of mutation groups

The absolute and relative risk were calculated for the pair-wise differences shown in Table 3.

Uses words	Absolute Risk Reduction (95% CI)	Relative Risk (95% CI)
R133C v. R168X	0.47 (0.18 -- 0.71)	14.5(1.9 -- 107.9)
R294X v. R168X	0.47 (0.20 -- 0.70)	14.5(2.0 -- 106.7)
other v.R168X	0.37(0.17 -- 0.53)	11.8(1.6 -- 83.9)
c-terminal truncations v. R168X	0.67 (0.40 -- 0.84)	20.5 (2.9 -- 143.9)
c-terminal truncations v. R306C	0.61 (0.30 -- 0.79)	7.4(1.9 -- 28.7)
c-terminal truncations v. large deletions	0.59 (0.26 -- 0.77)	6.0(1.6 -- 22.9)
Walks alone		
c-terminal truncations v. R168X	0.55 (0.25 -- 0.72)	3.0(1.6 -- 5.6)
R294X v. R168X	0.58 (0.26 -- 0.75)	3.1 (1.7 -- 5.8)
Uses hands		
R133C v. R168X	0.54 (0.21 -- 0.70)	2.4(1.5 -- 4.0)
c-terminal truncations v. R168X	0.50 (0.21 -- 0.68)	2.3(1.4 -- 3.8)
other v.R168X	0.40(0.17 -- 0.54)	2.1 (1.3 -- 3.4)

Table 5

Percentage of individuals with a specific mutation who retained function compared to those without.

Walks alone	With mutation (%)	Without mutation (%)	p-value	Absolute Risk Reduction (95% CI)	Relative Risk (95%CI)
R168X v. all other mutation groups	28	54	0.008	-0.26 (-0.41 -- -0.07)	0.5(0.1 -- 0.9)
R294X v. all other mutation groups	86	49	0.006	0.37(0.10 -- 0.49)	1.8(1.4 -- 2.3)
c-terminal truncations v. all other mutation groups	82	48	0.007	0.34(0.10 -- 0.48)	1.7(1.4 -- 2.2)
Uses hands					
R168X v. all other mutation groups	38	67	0.002	-0.29 (-0.46 -- -0.10)	0.6(0.1 -- 0.9)
Uses words					
R168X v. all other mutation groups	3	33	0.001	-0.30 (-0.37 -- -0.15)	0.1 (0.01 -- 0.7)
c-terminal truncations v. all other mutation groups	71	26	<0.001	0.45 (0.20 -- 0.62)	2.7 (2.3 -- 3.9)