Original Article

Does Genotype Predict Phenotype in Rett Syndrome?

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ABSTRACT

Mutations in the X-linked gene encoding the methyl-CpG binding protein MeCP2 are the primary cause of classic and atypical Rett syndrome and have recently been shown to contribute to other neurodevelopmental disorders of varying severity. To determine whether there are molecular correlates to the phenotypic heterogeneity, numerous groups have performed genotype-phenotype correlation studies. These studies have yielded conflicting results, in part because they used different criteria for determining severity and classifying mutations. Evolution of the phenotype with age and variable expressivity arising from individual variability in X-chromosome inactivation patterns are among other reasons the findings varied. Nonetheless, evidence of differences in the phenotypic consequences of specific types of mutations is emerging. This review analyzes the available literature and makes recommendations for future studies. (J Child Neurol 2005;20:768–778).
Mutations in the X-linked MECP2 gene are the primary cause of classic and atypical Rett syndrome (Online Mendelian Inheritance in Man 312750). In addition, this gene is implicated in a host of other neurodevelopmental disorders of varying severity affecting both female and male individuals. To determine whether the mutation contributes to variability in clinical presentation, numerous groups worldwide have performed genotype-phenotype correlation studies using cohorts of patients with classic and atypical forms of Rett syndrome. In this review, we provide an overview of these data and suggest steps that can be taken in future studies to clarify the relationship between genotype and phenotype in MECP2-related disorders.

**MECP2 MUTATIONS LEAD TO DIVERSE PHENOTYPES IN FEMALE AND MALE PATIENTS**

Since the identification of MECP2 mutation in Rett syndrome, one of the more surprising discoveries has been the wide range of phenotypes arising from mutation in this gene. Notably, these include both male and female patients with neurodevelopmental disorders that in many cases appear to be quite different from Rett syndrome. Although these other disorders are rare relative to Rett syndrome, they provide important insight into the phenotypic consequences of mutation in MECP2.

**Rett Syndrome Phenotypes: Classic and Atypical Forms**

Classic Rett syndrome is a clinically defined diagnosis based on adherence to a set of criteria that describe the neurodevelopmental and behavioral profiles of the child, irrespective of mutation status. Formal diagnostic criteria for the atypical or variant forms of Rett syndrome have also been delineated for cases that vary in terms of age at onset (congenital and late regression variants) or symptom severity (preserved speech andforme fruste variants).

Although the vast majority of patients meeting the criteria for Rett syndrome are girls, a growing number of boys with Rett syndrome have been reported; thus, female gender is no longer a criterion for diagnosis. Even within the constraints of the diagnostic criteria, classic and atypical forms of Rett syndrome demonstrate considerable variability in the presentation of specific signs and symptoms between patients and over time in the same patient. The evolution of phenotype with age is important to consider when evaluating the severity of the presentation of the disorder.

**Angelman Syndrome**

Angelman syndrome is a neurodevelopmental disorder caused by loss of the maternally expressed UBE3A gene on chromosome 15, either through deletion, paternal uniparental disomy, or an imprinting or intragenic mutation. The Angelman syndrome phenotype overlaps significantly with Rett syndrome, with gait abnormalities, ataxia, absent or greatly impaired language, seizures, and microcephaly present in both disorders. As such, distinguishing the two disorders clinically is sometimes difficult, and, perhaps not surprisingly, several patients diagnosed with Angelman syndrome (male and female) were found to have mutations in MECP2. Notably, these patients have normal chromosome 15 studies, and several were described as having some regression in skills, which is unusual for Angelman syndrome.

**Neonatal Encephalopathy**

MECP2 mutations have been identified in male and female patients presenting with several non–Rett syndrome phenotypes, including a severe encephalopathy that is not compatible with long-term survival. Most of these cases are boys born into Rett syndrome families, who presented in the neonatal period with central apnea, poor feeding leading to postnatal failure to thrive, microcephaly, seizures, and progressive spasticity or dystonia. Cognition and motor development are profoundly affected, and the children achieve few, if any, developmental milestones. In most cases, the brains appear to be structurally normal (although small); however, one case was noted to have polymicrogyria. It is important to note that no evidence exists indicating that MECP2 mutations are lethal prenatally in boys. Even in these most severely affected boys, prenatal growth and activity in utero were considered normal, yet they fail to transition to extrauterine life in the first few days and die in infancy or early childhood.

**Non–Rett Syndrome Mental Retardation**

A number of forms of mental retardation associated with neurologic or psychiatric symptoms or both have also been shown to arise from mutation in MECP2. These include families segregating nonsyndromic X-linked mental retardation in which there are multiple affected male members and normal or minimally affected carrier female members. The male members of these families frequently have mild-moderate mental retardation, although some are more severely impaired. Most of the patients are verbal but can have some degree of language delay or verbal stereotypies or both. Gross and fine motor skills are frequently impaired, and tremor, tone abnormalities, and seizures are also frequently reported. Male individuals have also been described with severe mental retardation, spasticity, and some Rett syndrome–like manifestations (Online Mendelian Inheritance in Man 300279), including absent speech, respiratory irregularities, and scoliosis. In some cases, the patients have had mental retardation with psychosis, pyramidal signs, and macro-orchidism (PPM-X syndrome; Online Mendelian Inheritance in Man 300055). One boy has been reported who manifested psychiatric symptoms and language disorder without significant cognitive impairment.

**Mutation Analysis in Rett Syndrome and Related Disorders**

The MECP2 gene spans 76 kb in chromosome Xq28 and is composed of four exons that undergo alternative splicing to generate two isoforms of the protein. Until recently, mutation analysis concen-
trated on exons 2 to 4, which encode the first isoform identified, MECP2A. The detection of mutations in exon 1 in several patients revealed a naturally occurring splice variant, MECP2B, which is generated from an alternative start site in exon 1, skips exon 2, and includes exons 3 and 4. These resultant proteins vary by only 37 amino acids at their N-termini. Most human tissues express both forms, with a modest predominance of MECP2A. A notable exception is brain in which the MECP2B splice variant is increased in abundance and shows regional and developmental regulation, becoming the primary isoform in adult human brain.

The identification of MECP2 mutations as the molecular basis of Rett syndrome catalyzed intense mutational screening of MECP2 in a number of disorders. As more patient data have accumulated and mutation analysis strategies have evolved to include examination of all four exons and conserved regions of the 5´ and 3´ untranslated regions, as well as the application of methods to detect large deletions and rearrangements, numerous new mutations have been revealed. To date, more than 175 different pathogenic mutations in MECP2 have been identified in classic and atypical cases of Rett syndrome, up from a mere 60 mutations in 2000. In sporadic cases of classic Rett syndrome, the likelihood of identifying a mutation in MECP2 approaches 95% (<http://www.bcmgeneticlabs.org/tests/alltests.html>) but decreases in the atypical cases. For atypical cases of Rett Syndrome, the reported efficiency of mutation detection varies considerably (range 25–75%), based on the criteria used for diagnosis, the size and age of the sampled population, and the sensitivity of the screening method. The lower incidence of MECP2 mutations in patients with atypical Rett syndrome also suggested the involvement of other gene(s) in this disorder, and, recently, mutations in the CDKL5 gene have been shown to result in a clinical phenotype overlapping Rett syndrome.

Nine recurrent point mutations account for 67% of mutation-positive cases of Rett syndrome (data from Rettbase; <http://mecp2.chw.edu.au/>) (Figure 1). These common mutations, as in most mutations found in Rett syndrome, are paternally derived cytosine to thymidine transitions, which typically arise de novo as a consequence of oxidative deamination of methylated CpG dinucleotides. In addition, approximately 10% of patients with Rett syndrome have small deletions in the 3´ end of exon 4 and another 10% carry large deletions and rearrangements. Missense mutations tend to cluster in the methyl binding domain, whereas nonsense and frameshift mutations are primarily located in the transcription repression domain and carboxy-terminus. Following the identification of the MECP2B splice variant in early 2004, mutation studies directed specifically toward exon 1 indicate that mutations in this exon are relatively rare; nonetheless, the notable absence of mutations in exon 2 in patients with Rett syndrome suggests that the MECP2B isoform is more relevant to the pathogenesis of the disorder.

Numerous frameshift mutations have been identified that affect the carboxy-terminus of the protein in which a WW binding domain has recently been described, suggesting a new role for MECP2 in binding to ribonucleic acid (RNA) splicing factors. This region (nucleotides 384–393) includes two simple direct repeats of four cytosines, making it susceptible to small deletions and insertions, leading to a high frequency of frameshift mutations. Because these mutations fall in the last exon of the gene, the transcripts are predicted to bypass nonsense-mediated decay pathways and generate proteins that have a missing or disrupted WW binding domain. Because this is a newly defined structural motif in MECP2, little is known about the role of MECP2 in RNA splicing and whether the dysfunction imposed by these mutations involves splicing in vivo.

Other MECP2-Based Neurodevelopmental Disorders

Mutation analyses of MECP2 in boys presenting with a variety of phenotypes have demonstrated several types of mutation. Boys presenting with the severe neonatal encephalopathy typically are hemizygous for mutations that cause Rett syndrome in girls, including missense mutations in the methyl binding domain and frameshift mutations in the transcriptional repression domain. Similarly, Rett syndrome mutations have been identified in boys present with classic or atypical Rett syndrome who were either mosaic for the mutation or had coexistent Klodefelter syndrome. However, recently, a boy was reported with a Rett syndrome phenotype who carries a de novo frameshift mutation (816dup7; P272fsX31X) and shows no evidence for mosaicism or X-chromosome aneuploidy. It is unclear why this mutation had less severe consequences than those found in boys with the congenital encephalopathy, but the amino acids encoded after the frameshift are unique and might allow better preservation of function or stability.

One mutation, A140V, has been identified in several unrelated families segregating X-linked mental retardation mapped to chromosome Xq28. This is a relatively conservative substitution of an alanine residue in the methyl binding domain; however, in vitro analyses of the mutant protein indicate that it leads to a modest change in DNA binding and transcriptional repression abilities. Girls carrying the A140V allele are either normal or mildly affected and do not typically show skewed X-chromosome inactivation. This mutation was also identified in a boy with childhood-onset schizophrenia and language disorder, prompting the investigation of MECP2 in a larger group of patients with schizophrenia, attention-deficit hyperactivity disorder, bipolar illness, alcoholism, puerperal psychosis, and phobia. These studies revealed a novel missense mutation in a female patient with schizophrenia (T196S); however, additional studies are needed to determine whether this is actually a susceptibility allele or a rare polymorphism.

A number of other mutations were identified in mentally retarded male patients, and it was initially suggested that MECP2 could be a major contributor to X-linked mental retardation. However, the frequency of mutations appears to be low based on several studies of relatively large patient cohorts. In addition, it appears that some of the mutations identified in mentally retarded male patients are actually rare polymorphisms and not pathogenic.

Mutation screening of MECP2 has been undertaken in patients with several neuropsychiatric disorders, with a particular focus on autism. Several studies of relatively large numbers of autistic patients did not reveal coding sequence mutations, suggesting that mutations in MECP2 are not a common cause of autism. However, a number of studies have found pathogenic mutations, including recurrent Rett syndrome mutations (R133C, R294X, L100V) and a novel missense mutation (P376R), in girls with autism with regression. In addition, screening of the conserved 3´ untranslated region identified a higher than expected number of sequence variants in an autistic subgroup. In two additional studies that included untranslated regions of the gene, three coding errors that
are predicted to be mild based on their carboxy-terminal location or conservative amino acid substitution, one splice site variant creating a small deletion, and two 3′ untranslated region changes were identified in autistic patients.56.68 Although the role of the 3′ untranslated region of MECP2 is yet to be elucidated, it is extraordinarily well conserved, raising the possibility that the variants contribute to the pathogenesis of these phenotypically related disorders.

**Molecular Influences on Variable Expressivity**

The basis of the variable expressivity for MECP2 mutations is likely to involve a complex interplay of several factors, including the level of residual function of the mutant protein, X-chromosome inactivation patterns, genetic background effects, and possibly environmental factors. In vitro analyses of mutant forms of MECP2 have shown a broad range of function of the proteins, with some mutations acting as null alleles that are incapable of binding to methylated target sequences and ineffective at repressing transcription from reporter constructs (eg, R106W). Many mutations (eg, T158M) lead to intermediate function of the protein in vitro, whereas some mutants appear similar to wild type in these assays (eg, R133C). Since it is clear that these mutations can cause Rett syndrome, failure to detect dysfunction of MECP2 in vitro demonstrates the limitations of the methods available to assay the complex function of this protein.

In boys with Klinefelter syndrome and girls, individual variation in X-chromosome inactivation patterns contributes to the differences in clinical presentation, particularly at the extreme ends of the phenotypic spectrum. Nonpenetrance of pathogenic mutations owing to skewed or nonrandom X-chromosome inactivation was originally identified in the nonmanifesting carriers in familial cases of Rett syndrome; however, subsequent studies have identified mutations and fortunate skewing of X-chromosome inactivation in several mothers of “sporadic” cases of Rett syndrome, a finding that has important clinical implications because it dramatically affects recurrence risk in these families.56.74.82.83

Studies of X-chromosome inactivation in patients with Rett syndrome that were done before MECP2 was identified as the causative gene revealed that most patients have essentially random patterns, with a slight tendency toward inactivation of the paternal allele. More recent studies that included both mutation analyses and X-chromosome inactivation studies identified a subset of patients with skewed inactivation, which in some cases clearly contributed to nonpenetrance of the mutant allele. To investigate the relationship between X-chromosome inactivation and phenotypic severity, Weaving and colleagues presented X-chromosome inactivation data on 72 patients with Rett syndrome in conjunction with mutation studies. They found that skewing was more prevalent than expected in their cohort of patients with Rett syndrome: 31 patients had skewing > 75%. The predilection for skewing was not clearly correlated with a specific type of mutation or with phenotype. This result does not mean that X-chromosome inactivation does not influence phenotype; instead, it illustrates the difficulties in interpreting X-chromosome inactivation data from peripheral tissues in a primarily neurologic disease. Individual variability of specific aspects of the phenotype is likely to reflect regional variation in expression of the wild-type or mutant protein in brain, which cannot be measured in a living patient. Thus, in most cases of Rett syndrome, examination of X-chromosome inactivation is not likely to be helpful for understanding the individual variation in presentation. In contrast, examination of X-chromosome inactivation is indicated for patients with overall very mild (or absent) phenotypes, as well as in patients who present with unusually severe phenotypes.

It is likely that genetic background and environmental factors also contribute to the phenotype in Rett syndrome, although these are poorly understood at present. In mice with targeted mutation of MECP2, strain effects on growth and weight gain support a
genetic background effect. In addition, differences in age at onset and rate of progression of symptoms in the inbred knockout mice suggest that other factors can influence phenotypic expression of the mutant allele.

Does Genotype Predict Phenotype in Rett Syndrome and Other MECP2-Related Disorders?

Given the wide range of phenotypes and multitude of mutations that have been identified in MECP2, the question arises as to how well genotype correlates with phenotypic outcome. This question should be considered at two levels: (1) Within the constraints of a clinical diagnosis of classic or atypical Rett syndrome, are specific mutations more likely to lead to specific deficits or a more severe overall presentation? and (2) Across the wide range of MECP2-related phenotypes, are specific mutations more likely to lead to Rett syndrome versus another disorder? Numerous genotype-phenotype correlation studies have been performed to determine how well specific mutation groups correlate with the overall severity of the phenotypic outcome, generally examining patients with a diagnosis of either classic or atypical Rett syndrome (Table 1). Cross-study comparisons are complicated, however, by a number of factors, including variability in assessment tools used to determine severity within the Rett syndrome and atypical Rett syndrome cohorts, which extended so far as to include variability in the definition of classic Rett syndrome. In addition, differences in how mutations were classified for the analyses (missense/nonsense/frameshift, by position of the mutation within the gene/protein, and by specific protein domain affected) further confound cross-study comparisons. Combined with the fact that in both Rett syndrome and atypical Rett syndrome the age of the subjects plays an important role in the presentation of symptoms (eg, scoliosis is more prevalent in older patients) and that an interrelationship between various components of the phenotype exists (eg, nonambulatory patients are more likely to have scoliosis), clearly determining specific genotype-phenotype correlations is not a trivial task. Nonetheless, evidence of some correlation is emerging, particularly in terms of the effect of specific mutations in the overall severity of the clinical presentation in the Rett syndrome–related phenotypes, as well as the association of particular types of mutations with the non–Rett syndrome phenotypes.

Are Missense Mutations Less Severe Than Nonsense Mutations?

One of the first reported genotype-phenotype correlations was that patients with Rett syndrome and atypical Rett syndrome with nonsense mutations were more severely affected than those with missense mutations. In this study, they used a relatively uncomplicated 3-point scoring system to assess phenotype in 24 patients with truncating mutations compared with 20 patients with missense mutations and detected a significant correlation that included correction for multiple testing ($P = .0023$). A correlation of more severe phenotypes with nonsense mutations was subsequently supported by studies from a number of other groups using more complicated 3-point scoring system to assess phenotype in 24 patients that have been identified in MECP2.

In the course of the mutation analyses, many groups have included patients diagnosed with either the forme fruste or the preserved speech variant of Rett syndrome, both of which are on the milder end of the spectrum of Rett syndrome–related phenotypes. Review of the mutations found in these 45 patients provides insight into genotype-phenotype correlations because the distribution of MECP2 mutations found in these patients is different from the distribution of mutations found in classic Rett syndrome (Table 2). Several of these mutations have been associated with lower severity scores and better adaptive responses in female patients diagnosed with classic and atypical Rett syndrome as well, suggesting that they are likely to be milder alleles. For example, in our study of 85 patients with classic or atypical Rett syndrome (none of whom met the criteria for a specific variant), the composite severity scores for patients with the R306C mutation were significantly lower than those of other mutation groups, and exclusion of those patients from the missense group eliminated significance for the missense-nonsense comparison. The most frequent mutations encountered in the milder variants are carboxy-terminal truncations, which are predicted to retain much of the normal function in that neither the methyl binding domain nor the transcriptional repression domain is disrupted. In keeping with this observation, the two studies that showed that nonsense mutations were more severe excluded mildly affected patients with late truncating mutations from the analyses. The other common mutation among the milder Rett syndrome variants is the R133C allele. A study that specifically examined patients carrying this mutation indicated that they function better as a group when compared...
<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients in Genotype-Phenotype Study (Diagnostic Groups)</th>
<th>Mutation Groups Examined (n)</th>
<th>Mutation Groupings Reported</th>
<th>Correlations</th>
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<tbody>
<tr>
<td>Amano et al, 2000&lt;sup&gt;97&lt;/sup&gt;</td>
<td>19 patients</td>
<td>6 missense, 13 truncating; 8 methyl binding domain, 11 transcriptional repression domain</td>
<td>Type, domain affected</td>
<td>Patients with mutations in or close to the methyl binding domain were more severely affected compared with patients with mutations in transcriptional repression domain or carboxy-terminus</td>
</tr>
<tr>
<td>Amir et al, 2000&lt;sup&gt;88&lt;/sup&gt;</td>
<td>48 patients (classic RTT)</td>
<td>18 missense, 30 truncating</td>
<td>Type</td>
<td>No correlation between composite severity score (13 features) and type of mutation</td>
</tr>
<tr>
<td>Bienvenu et al, 2000&lt;sup&gt;89&lt;/sup&gt;</td>
<td>46 patients (classic RTT)</td>
<td>8 missense, 21 truncating, 1 other; 16 no mutation detected</td>
<td>Type, domain affected; presence or absence of mutation</td>
<td>No correlation between severity of disease and type of mutation or domain affected; no significant differences between patients with and without detectable MECP2 mutation</td>
</tr>
<tr>
<td>Cheadle et al, 2000&lt;sup&gt;90&lt;/sup&gt;</td>
<td>64 patients (59 classic RTT; 5 nonclassic RTT-like)</td>
<td>21 missense, 24&lt;sup&gt;+&lt;/sup&gt; truncating, 17 no mutation detected; 10 early truncating before transcriptional repression domain, 16 late truncating (within or after transcriptional repression domain)</td>
<td>Type, position</td>
<td>Lower composite severity score (3 features) associated with missense mutations; early truncating mutations were more severe than late truncating mutations</td>
</tr>
<tr>
<td>Huppke et al, 2000&lt;sup&gt;91&lt;/sup&gt;</td>
<td>31 patients (26 classic RTT; 3 PSV; 1 congenital-onset RTT)</td>
<td>9 missense, 15 truncating, 7 no mutation detected</td>
<td>Type</td>
<td>No correlation between composite score (3 features) delineating function at age 5 yr and mutation type</td>
</tr>
<tr>
<td>Auranen et al, 2001&lt;sup&gt;92&lt;/sup&gt;</td>
<td>40 patients (39 classic RTT, 1 PSV)</td>
<td>19 missense, 21 truncating</td>
<td>Type, mutation</td>
<td>No significant correlation between mutation type and severity; phenotypes were milder for patients with R294X (n = 4) and more severe for patients with R255X (n = 5), G269fsX (n = 2), and R270X (n = 4)</td>
</tr>
<tr>
<td>Giunti et al, 2001&lt;sup&gt;93&lt;/sup&gt;</td>
<td>75 patients (classic RTT)</td>
<td>22 missense, 42 truncating, no mutation detected</td>
<td>Type, mutation</td>
<td>No phenotype-genotype correlation detected</td>
</tr>
<tr>
<td>Hoffbuhr et al, 2001&lt;sup&gt;94&lt;/sup&gt;</td>
<td>44 patients (classic RTT)</td>
<td>19 missense upstream of or in methyl binding domain; 6 nonsense between methyl binding domain and transcriptional repression domain; 9 truncating in transcriptional repression domain; 5 missense in transcriptional repression domain; 6 frameshift deletions in carboxy-terminus</td>
<td>Combined position and type</td>
<td>Patients with early mutations (missense in the N-terminus or methyl binding domain) or truncations before the transcriptional repression domain had more severe phenotypes than those with late-occurring mutations (missense, nonsense, and frameshift in the transcriptional repression domain or carboxy-terminus; X-chromosome inactivation was related to severity of disease in patients with early mutations</td>
</tr>
<tr>
<td>Inui et al, 2001&lt;sup&gt;95&lt;/sup&gt;</td>
<td>20 patients (mostly atypical)</td>
<td>7 missense; 3 truncating; 10 no mutation detected</td>
<td>Presence or absence of mutation</td>
<td>No significant differences in presence of diagnostic criteria in patients with mutations compared with those without identifiable mutation</td>
</tr>
<tr>
<td>Monros et al, 2001&lt;sup&gt;96&lt;/sup&gt;</td>
<td>47 patients (35 classic RTT, 5 congenital onset, 4 PSV, 1 FF)</td>
<td>18 missense, 23&lt;sup&gt;+&lt;/sup&gt; truncating</td>
<td>Type, 23&lt;sup&gt;+&lt;/sup&gt; truncating</td>
<td>Significant difference detected between missense and truncating mutations, as assessed by sitting alone, ambulation, and age at onset criteria; missense mutations were associated with a milder phenotype based on 9-feature composite score</td>
</tr>
<tr>
<td>Nicolao et al, 2001&lt;sup&gt;97&lt;/sup&gt;</td>
<td>27 patients (classic RTT)</td>
<td>9 missense, 3 early truncating, 15 late truncating</td>
<td>Type, position of truncating mutations</td>
<td>No significant correlations detected</td>
</tr>
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</table>
Table 1. (continued) Summary of Genotype-Phenotype Studies Performed in Patients With Rett Syndrome or Other MECP2-Related Disorders

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of Patients in Genotype-Phenotype Study (Diagnostic Groups)</th>
<th>Mutation Groups Examined (n)</th>
<th>Mutation Groupings</th>
<th>Reported Correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamada et al, 200101</td>
<td>37 patients (14 classic RTT, 13 atypical RTT, 10 RTT-like)</td>
<td>9 missense, 17 truncating, 12 no mutation detected</td>
<td>Type; presence or absence of mutation</td>
<td>No significant correlations detected</td>
</tr>
<tr>
<td>Huppke et al, 200202</td>
<td>123 mutation positive RTT</td>
<td>50 missense, 73 truncating; 40 methyl binding domain, 29 transcriptional repression domain, 23 nuclear localization signal</td>
<td>Type, position, domain affected (methyl binding domain, transcriptional repression domain, nuclear localization signal)</td>
<td>Higher composite severity scores (6 features at age 5 yr) for patients with truncating mutations compared with missense and carboxy-terminal truncations; no difference in methyl binding domain vs transcriptional repression domain; patients with nonsense mutations in the nuclear localization signal were most severe</td>
</tr>
<tr>
<td>Gomot et al, 200303</td>
<td>13 males in 3 families with MRX caused by MECP2 mutations</td>
<td>In-frame deletion in the 3’ region, R167W, E137G</td>
<td>Mutation between and within families</td>
<td>Phenotypic heterogeneity was apparent in males in all families; carrier females had normal phenotypes; skewed X-chromosome inactivation in one female with in-frame deletion</td>
</tr>
<tr>
<td>Leonard et al, 200304</td>
<td>122 patients</td>
<td>24 R133C, 98 other</td>
<td>R133C compared with other mutations</td>
<td>Patients with R133C exhibit better overall function, without evidence for skewed X-chromosome inactivation</td>
</tr>
<tr>
<td>Weaving et al, 200305</td>
<td>138 patients; 35–68 patients included in phenotyping studies</td>
<td>57 missense, 81 truncating</td>
<td>Type, domain affected</td>
<td>No significant difference in overall severity between missense and truncating mutations, although more severe subscres for head growth, somatic growth, speech, and hand use for patients with nonsense mutations</td>
</tr>
<tr>
<td>Chae et al, 200206</td>
<td>27 patients (classic RTT)</td>
<td>12 missense, 15 truncating</td>
<td>Type</td>
<td>No significant difference in overall severity between missense and truncating mutations</td>
</tr>
<tr>
<td>Colvin et al, 200407</td>
<td>109 patients (51 classic, 58 atypical)</td>
<td>44 missense, 63 nonsense, 2 unclassified; 33 methyl binding domain, 15 interdomain, 22 transcriptional repression domain—nuclear localization signal, 20 transcriptional repression domain—after nuclear localization signal, 14 carboxy-terminal deletions; 3 R106W, 9 R133C, 14T158M, 13 R168X, 8 R255X, 11 R270X, 9 R284X, 5 R306C</td>
<td>Type; domain affected; mutation</td>
<td>More severe phenotype associated with nonsense mutations, particularly in the nuclear localization signal region; truncations after nuclear localization signal were less severe; R133C and R294X were least severe</td>
</tr>
<tr>
<td>Schanen et al, 200408</td>
<td>85 patients (74 classic RTT, 11 atypical)</td>
<td>36 missense, 49 nonsense; 28 methyl binding domain, 40 transcriptional repression domain; 3 early truncating, 26 missense upstream of or in methyl binding domain, 11 nonsense between methyl binding domain and transcriptional repression domain, 29 truncating in the transcriptional repression domain, 10 missense in transcriptional repression domain, 6 frameshift in carboxy-terminus; 17T158M, 10R168X, 12R255X, 8 R284X, 9 R306C</td>
<td>Type, domain affected, combined, mutation</td>
<td>Decreased severity based on composite score (13 features) for patients with the R306C mutation; patients with missense mutations had a less severe overall phenotype than patients with nonsense mutations; however, the difference was driven by the patients with the R306C mutation; age at onset of regression and head growth correlate with severity of the disease</td>
</tr>
</tbody>
</table>

FF = forme fruste; MRX = Mental Retardation X-linked; PSV = preserved speech variant; RTT = Rett syndrome.
The number of patients included in the correlation study is noted, which is frequently lower than the total number of patients in the mutation studies. The number of subjects in the groups that were compared is noted.
Study excluded two patients with late truncating mutations from the missense-nonsense comparison.
1Study excluded five patients with carboxy-terminal truncations from the analyses.
2Study uses some of the same patients as Huppke et al, 2000.91
with patients with other mutations. Notably, although these mutations can be more frequent in the milder forms of Rett syndrome, each of them has also been identified in moderately to severely affected patients. Thus, one must remain cognizant of the limitations of using mutation data to predict phenotype. Indeed, it appears that the converse prediction can be made more reliably; mutation analysis for a patient with a mild phenotype is likely to disclose either a carboxy-terminal truncation or one of a small group of missense mutations.

**Genotype-Phenotype Correlations in Hemizygous Male Patients**

The phenotypic manifestations of MECP2 mutations in male patients can be broadly classified into three groups that range widely in severity, from neonatal-onset encephalopathy to mild mental retardation. Although the specific impact of the mutation on phenotype is somewhat more apparent in hemizygous males, genotype-phenotype correlations are not entirely straightforward. Heterogeneity of expressivity of the same mutation within and among families has also been noted, leading to debate over the pathogenicity of mutations identified in males.

Nonetheless, available data suggest that boys who carry mutations that typically lead to Rett syndrome in girls often present with a severe neonatal-onset encephalopathy that is associated with death in infancy or early childhood. In contrast, the mutations that are found in boys with mental retardation are not observed in girls with Rett syndrome and tend to be conservative mutations that fall outside the known functional domains or are missense mutations in the carboxy-terminus. The carboxy-terminal truncations that cause Rett syndrome or atypical Rett syndrome in girls have also been identified in boys presenting with moderate to severe mental retardation. The mildest male phenotype reported arose from an in-frame deletion of amino acids 388 to 467 in a family with nonsyndromic X-linked mental retardation (MRX).

**Phenotypic Heterogeneity Within Mutation Groups**

The most consistent finding in each of the genotype-phenotype studies done to date is that regardless of the clinical scoring system used or the criteria by which the mutations are grouped, tremendous heterogeneity exists for the phenotypes manifested by patients within the same group, even for related patients with the same mutation. This consistent inconsistency in phenotypic manifestations of mutation in MECP2 is important to keep in mind because it means that it is not possible to predict phenotypic outcome for any single patient based solely on mutation data.

**Future Directions**

After 5 years of mutation analyses of the MECP2 gene in patients with Rett syndrome and other neurodevelopmental disorders, we have gained some insight into the molecular basis of the clinical variability, although many studies have failed to identify correlations or have reported conflicting correlations. These conflicting results are likely to arise from a combination of factors, including grouping of specific mutations that have very different effects on residual function of MECP2, wide variation in the age of the subjects in the studies, sampling bias from relatively small cohorts and underpowered studies, failure to take multiple testing into account, leading to type I errors, and variability in the clinical measures being assessed.

It is likely that investigators will continue to use a variety of severity scoring systems to assess phenotype. As such, a number of points need to be considered in the conversion of the clinical data into a severity score. These points are mentioned below.

**Weight of All Measured Parameters**

The idea of identically weighing all parameters is of interest. For example, should more severe microcephaly have a greater impact on the score than the frequency and persistence of stereotypic hand movements? At this point, it is unclear whether any specific aspect of the Rett syndrome phenotype directly correlates with outcome. Similarly, the scoring range should be the same for each of the component scores. This confounding factor became apparent in the application of the scoring system that we derived to assess the phenotypes in our cohort of patients and necessitated steps to normalize the component scores to prevent inappropriate weighting of the composite score by subscores with larger numbers of categories.

**Scoring Signs or Symptoms That Are Transient or Resolve**

Should the scoring system include internal corrections for age? If a large enough sample of phenotypic data is collected, including longitudinal data, then age-matching strategies can be applied. This was done in the studies by Huppke and colleagues, who examined the function of the children at age 5 years.

**Effect of Interaction Among Various Phenotype Aspects**

Although it is intuitive that various aspects of the Rett syndrome phenotype are likely to be etiologically related (e.g., prolonged Q–Tc interval, breathing irregularities, and peripheral autonomic dysfunction might reflect brainstem involvement) or that one type of dysfunction might affect the severity of another component

### Table 2. Summary of Mutations Reported in Mild Rett Syndrome Variants

<table>
<thead>
<tr>
<th>Mutation (number reported; frequency)</th>
<th>Phenotype (n)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E100Q (1; 2.2%)</td>
<td>Forme fruste</td>
<td>101</td>
</tr>
<tr>
<td>P127L (1; 2.2%)</td>
<td>PSV</td>
<td>102</td>
</tr>
<tr>
<td>R133C (9; 20%)</td>
<td>Forme fruste (3), PSV (6)</td>
<td>38, 66, 100, 101, 103–105</td>
</tr>
<tr>
<td>T158M (2; 2.2%)</td>
<td>Forme fruste (1), PSV (1)</td>
<td>105</td>
</tr>
<tr>
<td>T158A (1; 2.2%)</td>
<td>PSV</td>
<td>105</td>
</tr>
<tr>
<td>R168X (1; 2.2%)</td>
<td>PSV</td>
<td>38</td>
</tr>
<tr>
<td>P302A (1; 2.2%)</td>
<td>PSV</td>
<td>38</td>
</tr>
<tr>
<td>R306C (6; 11.1%)</td>
<td>Forme fruste (1); PSV (4)</td>
<td>101, 104, 105</td>
</tr>
<tr>
<td>*Carboxy-terminal deletions (23; 51.1%)</td>
<td>Forme fruste (7); PSV (16)</td>
<td>100, 101, 104–106</td>
</tr>
<tr>
<td>Duplication (1; 2.2%)</td>
<td>PSV</td>
<td>107</td>
</tr>
<tr>
<td>Total: 45 cases</td>
<td>Forme fruste (12) PSV (32)</td>
<td></td>
</tr>
</tbody>
</table>
score (eg, increased scoliosis in nonambulatory patients), in most cases, the interrelationships of these parameters have not been statistically tested. Thus, when analyzing component score clinical data sets, it is unclear which variables need to be covaried to best characterize the specific aspects of the phenotype.

Methods for Handling Missing Data
In the course of collecting retrospective clinical data, it is inevitable that data sets will be incomplete. This is particularly a problem for scoring systems that are simply additive composite scores and can be rectified in part by using mean severity scores.

None of the current severity scoring systems encompass non–Rett syndrome phenotypes, and application of the measures designed for patients with Rett syndrome might not be helpful for determining the relative severity of the non–Rett syndrome disorders associated with MECP2 mutations.

Rigorous Statistical Approaches to Avoid Type I and Type II Errors
Large samples are needed to overcome the wide range of heterogeneity of the phenotypes in classic and atypical Rett syndrome because small samples are prone to both type I and type II errors arising from artifacts in sampling. Investigators need to remain vigilant in terms of correcting for multiple testing, particularly when the phenotype subscores are evaluated individually or multiple mutation groupings are used in the analyses or both.

Ultimately, it is likely that on-line data collection will help rectify the problems with these types of studies. Given the age effect on phenotype, optimally, the clinical data collected will include both a cross-sectional view of a large population of patients and longitudinal data on the patients over time. Two on-line databases have been developed to help correlate the diverse longitudinal data on the patients over time. Two on-line databases have been developed to help correlate the diverse longitudinal data on the patients over time. Two on-line databases have been developed to help correlate the diverse longitudinal data on the patients over time. Two on-line databases have been developed to help correlate the diverse longitudinal data on the patients over time. Two on-line databases have been developed to help correlate the diverse longitudinal data on the patients over time.

Currently, the InterRett database allows collection of categorical data on various aspects of the Rett syndrome phenotype, which can be converted into a severity score. It is hoped that the application of a uniform set of phenotypic severity scores for a large number of patients will help clarify the conundrums of genotype-phenotype correlations in Rett syndrome and other related phenotypes.

References


