THE PHENOTYPIC CONSEQUENCES OF MECP2 MUTATIONS EXTEND BEYOND RETT SYNDROME

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Although MECP2 was initially identified as the causative gene in classic Rett syndrome (RTT), the gene has now been implicated in several phenotypes that extend well beyond the clinically defined disorder. MECP2 mutations have been found in people with various disorders, including neonatal onset encephalopathy, X-linked recessive mental retardation (MRX), classic and atypical RTT, autism, and Angelman syndrome, as well as mildly affected females and normal carrier females. To make matters more complex, in approximately 20% of classic sporadic RTT cases and more than 50% of affected sister pairs, no mutation in MECP2 has been found. X-chromosome inactivation patterns can clearly affect the phenotypic expression in females, while the effect of the type and position of the mutation is more apparent in the broader phenotype than in RTT. Both males and females are at risk, although an excess of paternally derived mutations are more apparent in the broader phenotype than in RTT. Both males and females are at risk, although an excess of paternally derived mutations are found in most cases of classic RTT. Thus, because of the range of disparate phenotypes, the gene may account for a relatively large portion of mental retardation in the population.

Key Words: Rett syndrome; MECP2; Angelman; X-linked mental retardation

As a neurodevelopmental disorder that most commonly strikes females, Rett syndrome (RTT) has been an enigmatic disorder since it was first recognized. The identification of mutation in MECP2 as the cause of most cases of RTT affords us the opportunity to explore the mechanisms that underlie the disorder both clinically and molecularly. However, the gene involved is also proving to be a bit more complex in terms of the heterogeneous manifestations of mutation. In examination of the broader phenotypes resulting from MECP2 mutations, the expressivity of the mutant allele in heterozygous females with balanced X-inactivation patterns indicates that some alleles show X-linked recessive effects, while the mutations found in RTT are dominant acting. These differentially acting alleles also manifest as various phenotypes in males, as males that are hemizygous for RTT causing MECP2 mutations present with a severe neonatal encephalopathy, while males with recessive-acting mutations exhibit nonspecific X-linked mental retardation (MRX) phenotypes. In short, mutation in MECP2 is not synonymous with RTT, and RTT is not always caused by an identifiable mutation in MECP2. While this may seem like semantics, it is important to distinguish the clinical diagnosis of RTT because there are data for complications and prognosis for individuals with the more classical presentations that cannot be applied across the broader phenotypes of those with MECP2 mutation. Finding connections between MECP2 mutation type, penetrance, and phenotype in both males and females will perhaps elucidate the mechanism by which mutation in MECP2 can cause several different forms of neurodevelopmental dysfunction.

SPECTRUM OF MECP2 MUTATIONS IN CLASSICAL AND ATYPICAL RTT

In its classic form, RTT is generally a sporadic disorder that is recognized in about 1/15,000 females. Although familial cases of RTT occur, there are considerably more sporadic cases, which reflects the relatively high frequency of mutation of the human MECP2 gene. There is a propensity for C to T transitions, leading to nonsense and missense mutations, as well as small deletions and insertions in the coding sequence (Fig. 1). In classic cases, the rate of mutations identified approaches 80%–90% [Amir et al., 2000; Cheadle et al., 2000; Hoffbuhr et al., 2001], with lower rates in atypical cases (30%) [Hoffbuhr et al., 2001]. In almost all of the familial cases used to map the locus, mutations have been identified, however, only about 20 %–25% of sister pairs have identifiable mutations [Amir et al., 2000]. In karyotypically normal boys with RTT phenotypes, only a few have had mutations in this gene, although it is responsible for a plethora of other disorders in boys (see below). While it is possible that patients with RTT who do not have mutations in the coding regions of MECP2 have novel noncoding mutations or large inversions or rearrangements that escape PCR-based screening strategies, it seems likely that a second disease locus exists that could contribute to some cases of RTT and atypical
RTT. Whether this locus is X-linked is unclear; however, the identification of totally skewed XCI in some families with recurrent RTT raises the possibility of a second gene on Xp [Villard et al., 2000].

The dosage and mutation type of MECP2 appear to contribute to disease phenotype, although, within the domain of classic and atypical RTT, specific genotype-phenotype correlations have had mixed results. In part, this stems from marked variability in the criteria used between groups to define clinical parameters, but also arises from variable expressivity of the same mutation in different probands because of regional differences in X-chromosome inactivation (XCI) patterns. Within the classic and atypical cases, nonsense truncating mutations have been associated with awake respiratory dysfunction and nonrandom XCI, while missense mutations have been linked with scoliosis [Amir et al., 2000]. Hoffbuhr et al. also reported that mutations lying in the N-terminus of MeCP2 correlated with a more severe phenotype than mutations in the carboxy end of the protein, measured by comparison of head growth deceleration in patients with different MECP2 mutations [2001].

THE INFLUENCE OF XCI ON MECP2 MUTATION EXPRESSION

The MECP2 gene is subject to XCI in females and, thus, the phenotypic expression of mutations is influenced by the relative activity of the normal and abnormal alleles in relevant tissues [Adler et al., 1995]. Although for some X-linked genes, mutation leads to secondary skewing of inactivation because of a selective advantage of cells expressing the normal allele, this does not appear to be the case for most mutant forms of MECP2. Numerous studies of XCI patterns in RTT indicate that most probands have random inactivation patterns in blood, skin, and brain [Vorsanova et al., 1996; Webb and Watkiss, 1996; Zoghbi et al., 1990]. Nonetheless, for girls with classic or atypical RTT resulting from MECP2 mutation, it is likely that the individual variability in symptoms stems from regional differences in the XCI patterns in the nervous system, although this is difficult—if not impossible—to prove at this juncture. Fortunately skewing of inactivation, defined as > 80% inactivation of the chromosome carrying the mutant gene, has clearly been shown to have an ameliorating effect on the phenotype in several normal or mildly affected carrier females [Amir et al., 2000; Bienvenu et al., 2000; Schanen et al., 1997]. Most importantly, this includes mothers of seemingly sporadic cases of RTT. Although it is possible that the skewed inactivation results from selection against the mutant allele, in each of the known cases of asymptomatic carriers with fortunate XCI, an affected family member with the same mutation has a random pattern [Schanen et al., 1997; Sirianni et al., 1998; Wan et al., 1999]. Similarly, there is limited evidence suggesting that early truncating mutations have deleterious effects at the cellular level leading to secondary skewing of inactivation; however, these cases were also ascertained because of the existence of a relative with RTT and balanced XCI. If there is no selection against the mutant allele, presumably, unfortunate inactivation could also occur, but may result in symptoms that are not recognized as being related to RTT (see below).

Recent studies indicate that MECP2 mutations manifest in a broader clinical presentation than those encompassed under the RTT spectrum (Fig. 2). Variability in expressivity of MECP2 mutations in females is expected because of the influence of XCI; however, there are growing data indicating that the effect of mutation in males is equally complex. The range of phenotypes in boys who are hemizygous for MECP2 mutations extends from a neonatal onset-encephalopathy associated with early death to several forms of non syndromic X-linked recessive mental retardation compatible with long-term survival [Schanen et al., 1998; Clayton-Smith et al., 2000; Meloni et al., 2000; Orrico et al., 2000; Villard et al., 2000; Couvert et al., 2001; Hoffbuhr et al., 2001; Imessaoudene et al., 2001]. In addition, RTT or RTT-like phenotypes
also occur in boys and may or may not result from MECP2 mutation. The extreme variability in phenotype likely results in many males with MECP2 mutations being overlooked or diagnosed with a neurodevelopmental disorder more typically seen in males, such as autism.

MALE PHENOTYPES

The first male phenotype clearly linked genetically to RTT was identified in boys born into kindreds with girls with classic RTT. In these cases, the boys were born with normal growth parameters following uneventful pregnancies and deliveries. They then presented with severe neurologic symptoms in the newborn period. Common symptoms included a static encephalopathy associated with profound developmental delays, hypotonia, seizure, acquired microcephaly, ataxia, and repeated face scratching movements that are reminiscent of hemizygosity for an MECP2 mutation.

A third neonatal encephalopathic patient with a truncating mutation has been identified who carries a P816X89 mutation, which causes a very early stop in the MBD [Clayton-Smith et al., 2000]. The mutation occurs before the third splice site, so it is very likely that the message undergoes nonsense mediated decay (NMD) rather than translating into a truncated protein. The patient’s phenotype is much less severe than those of the late-truncating mutations; he had some speech ability until the age of two, walks with an ataxic gait, is hypotonic, began experiencing seizures at age three, and was still alive at the age of six [Clayton-Smith et al., 2000]. The patient is somatic mosaic for this de novo P816X89 mutation, indicated by the presence of both a normal and mutant band in restriction digests of the MECP2 region [Clayton-Smith et al., 2000]. Somatic mosaicism is most likely responsible for the milder phenotype manifested by this male as compared with those with the neonatal encephalopathy phenotype. It is also possible that the absence of any MeCP2 protein is less detrimental than the presence of a truncated version that could possibly have a dominant negative effect.

RETT SYNDROME IN KLINEFELTER PATIENTS

Although the above patients with neonatal onset encephalopathy have mutations in the gene responsible for many cases of RTT, they do not meet the defined diagnostic criteria for RTT. However, there have been a few cases reported in which Klinefelter syndrome patients (karyotype 47, XXX) exhibit hallmark symptoms of RTT, including stereotypical hand movements, hypotonia, and a loss of acquired skills over an extended period of time [Vorsanova et al., 1996; Schwartzman et al., 1998; Leonard et al., 2001]. The presence of two X-chromosomes in Klinefelter males would lead to a similar situation as in females with RTT who are mosaic for the mutant allele. RTT occurs in about 1/10,000 female births, and the incidence of Klinefelter syndrome is approximately 1/1,000 male births, so Klinefelter and RTT are expected to coincide in only about 1/100 male births. For this reason, very few cases of Klinefelter patients with RTT have been reported. Schwartzman, et al. reported a sporadic case of RTT in a 47,XXX male who presented with stereotypical hand gestures, loss of purposeful hand movement and language skills, constipation, ataxia, and apnea, all after an eight-month period of normal development [1998]. The additional X-chromosome was found to be of paternal origin, and the proband’s XCI pattern determined to be random, although no MECP2 mutation was identified [Schwartzman et al., 1998]. Leonard et al. [2001] reported a mosaic Klinefelter RTT male (47,XXX[23]/46,XY[7]) in peripheral blood lymphocytes) with the T158M mutation in MECP2. The proband exhibited hand wringing movements, hypotonia, constipation, myoclonic seizures and gradual loss of speech and coordinated muscle movement. He is mentally retarded and wheelchair bound [Leonard et al., 2001]. Vorsanova et al. reported another 47,XXX/46,XY RTT male who was found to have an extra X-chromosome in 12% of his lymphocytes and presented with a classical RTT phenotype [1996]. Although only a few cases have been identified thus far, it is important to note that classical or atypical RTT resulting from MECP2 mutation can occur in boys who carry an additional X-chromosome, either constitutionally or in a mosaic form or who have a postzygotic mutational event leading to mosaicism of expression of the mutant allele.

There have also been a number of boys reported who meet many or all of the diagnostic criteria for RTT, but who are karyotypically normal [Coleman, 1990; Philippart, 1990; Eeg-Olofsson, 1999; Jan et al., 1999]. Results of MECP2 mutation analysis for most of these cases are not known; however, Hoffbuhr et al. screened nine boys in their cohort and did not find MECP2 mutations in any of them [Hoffbuhr et al., 2001]. In addition, one boy reported by Leonard et al. meets all of the clinical criteria for RTT but does not have a
detectable MECP2 mutation [2001]. Thus, as is the case with some RTT girls, there are boys who have clinical features of RTT, but have no detectable mutation in MECP2.

MECP2 MUTATION AND MRX IN MALES

The discovery of MECP2 not only answered the long search for the faulty gene in RTT, but also shed new light on the molecular defect underlying non-specific MRX. The prevalence of MRX is estimated as 1/600 to 1/300 in males [Herbst and Miller, 1980; Glass, 1991] and, therefore, it is considered a common cause of mental deficiency in males. The link between MECP2 and this heterogeneous group of disorders demonstrates that hemizygosity of an MECP2 mutation is not always lethal in males and could present as mental retardation or an array of other neurological disorders [Meloni et al., 2000; Orrico et al., 2000; Couvert et al., 2001; Imessaoudene et al., 2001].

Investigating the possibility of MECP2 mutations in families and cohorts of males and females with X-linked recessive mental retardation was triggered by the phenotypic variability of these mutations in RTT. Meloni et al. studied a three-generation family in which the gene was mapped to Xq27.2-qter, and candidate genes in the region were excluded previously [2000]. They identified a Q406X mutation in the uncle and nephew and their mothers. In contrast to RTT, both males were macrocephalic and had normal growth, diarhea, and retained purposeful hand skills. They were severely mentally retarded and never developed language. Facial hypotonia, sialorrhea, seizures, and ataxic gait were all evident. One male exhibited spasticity in all limbs and a choreothetoid movement in one hand while the other had hypertonia only in the lower limbs and bruxism. Despite a random X-inactivation pattern in blood leukocytes in both carrier females, one showed borderline intelligence and mild facial hypotonia while no physical and mental abnormalities were observed in the other [Claes et al., 1997].

In a second case of inherited MECP2 mutation, Orrico et al. [2000] identified a family in which four adult brothers with severe mental retardation were found to carry an A140V mutation. The maternally inherited mutation was also passed on to the only daughter of the family. The affected males presented as normocephalic with impaired expressive language, resting tremors, and slowness of movement. The females were shown to have mild MR, microcephaly, poor muscle build, and speech and gait difficulties [Orrico et al., 2000]. The A140V and five other mutations, R167W, E137G, P399L, R453Q, and K284E, were detected in seven other cases of isolated MRX where the common phenotypic presentation in the males was moderate to severe MR [Couvert et al., 2001]. In addition to MR, speech impairment, resting tremors, aggressive behavior, and psychiatric disturbances including auditory and visual hallucinations, insomnia and anxiety were noted in some of the patients. The probands' mothers were carriers of the mutation and had normal cognition and patterns of X-inactivation [Couvert et al., 2001]. Imessaoudene et al. [2001] also reported on the involvement of a G428R mutation in the MECP2 gene and nonprogressive encephalopathy in a male. Developmental delay, uncoordinated limb and trunk movements, agitation, and absence of language were the neurological disturbances reported. The mutation was shared with the normal mother and two maternal aunts who had balanced XCI patterns. Importantly, the pathogenicity of this mutation has not been established, although it has not been reported in unrelated normal controls. In addition, Imessaoudene et al. reported mutations in MECP2 in patients who carried the clinical diagnosis of possible Angelman syndrome but had normal chromosome 15 methylation patterns [Imessaoudene et al., 2001].

Although not lethal, similar to RTT, MECP2 mutations in MRX seem to affect primarily cognition and expressive language in the affected individuals. It is interesting to note that regression following a normal period of development, which is the hallmark characteristic of classical RTT, has not been reported in any of the cases. Surprisingly, XCI studies in all females did not reveal any abnormality; nonetheless, a non random pattern of X-inactivation in tissues other than blood is a plausible explanation for the abnormal phenotypes observed.

THE MOLECULAR BASIS OF VARIABLE EXPRESSION OF MECP2 MUTATIONS

The heterogeneity of phenotypes caused by mutation in MECP2 is best explained by the combined effect of XCI and the likelihood that different mutations have variable impact on the function of MeCP2. Based on available data, it appears that mutations that lead to classic RTT in females are most often associated with the neonatal encephalopathy phenotype in karyotypically normal males [Wan et al., 1999; Villard et al., 2000; Ben Zeev et al., personal communication]. These mutations are predicted to have a more deleterious effect on MeCP2 function than those found in MRX. The T158M mutation involves the substitution of a hydrophobic amino acid with a polar residue in a hairpin loop in the MBD of MeCP2. Although this residue is on the opposite face of the DNA binding domain, it is possible that it is involved in interactions with other portions of the MeCP2 molecule, thereby stabilizing the protein structure in relation to DNA [Free et al., 2001]. In fact, it has been found that the T158M mutation slightly reduces binding affinity to methylated oligonucleotides and heterochromatin and has moderate effects on transcriptional repression as well [Ballesta et al., 2000; Free et al., 2001; Kudo et al., in press]. Both the R268S/K288 and G252S/K258 mutations cause premature stops in translation in the TRD, most likely forming a truncated MeCP2 protein. A truncated protein would disrupt the TRD but leave the MBD intact, which could cause MeCP2 to act in a dominant negative manner by binding DNA but being unable to recruit the transcriptional repression complex. This could block methyl-CpG sites from being bound by other functional methyl-CpG binding proteins. Alternatively, binding of the MBD to DNA could induce partial repression, making these TRD stop mutations less severe than missense mutations occurring in the MBD. Loss of the carboxy-terminus of MeCP2 may also lead to protein instability, potentially rendering the mutations as functionally null alleles. Understanding which of these molecular mechanisms actually occurs will perhaps elucidate a connection between MECP2 mutation and the neonatal encephalopathy phenotype.

It is predicted that MRX-causing MECP2 mutations have a less deleterious effect on the MeCP2 protein than those causing RTT, given the less severe phenotypes observed in both males and females [Orrico et al., 2000; Couvert et al., 2001; Hoffbuhr et al., 2001]. The A140V and E137G mutations both lie in the α-helix of the MBD. The wedge shaped structure of the MBD is composed of four antiparallel β-sheets, which form one face of the wedge and are presumed to interact with methylated CpG dinucleotides in the major groove of DNA [Wakefield et al., 1999]. Common RTT causing mutations, such as R106W...
and R133C, lie in the region that forms this face of the wedge. The α-helix that harbors the A140V and E137G mutations forms the opposite side of the wedge, and is therefore not believed to directly interact with DNA [Ohki et al., 1999; Wakefield et al., 1999]. The A140V mutation is predicted to shorten the length of the α-helix by half, potentially having a mild effect on MeCP2 function by modifying the wedge shaped structure of the MBD [Or rico et al., 2000]. In fact, Kudo et al. demonstrated that the A140V mutation does not alter MeCP2 affinity for heterochromatin or transcriptional repressive activity by a methylated promoter, but results in greater repression from an unmethylated promoter [Kudo et al., personal communication]. This could result in nonspecific repression by MeCP2 in the case of the A140V mutation, and therefore a phenotype different from that of RTT. The less severe effects of the P399L, R453Q, G428S, and Q406X mutations are proposed to be caused by their positions in the C terminus of MeCP2, a histidine and proline rich region of the protein believed to facilitate nonspecific binding to DNA [Chandler et al., 1999; Couvert et al., 2001; Imessaudene et al., 2001; Meloni et al., 2000]. The R167W mutation locates to the region between the MBD and TRD, so a substitution here may not perturb MeCP2 function as much as a missense mutation lying directly inside the MBD or TRD.

CONCLUSIONS

The diverse range of phenotypes observed to be caused by mutation in MECP2 not only sheds new light on the possible consequences of MECP2 mutation at the molecular level, but has further ramifications in genetic counseling as well. It is important to consider that males can present with neurodevelopmental dysfunction as a result of MECP2 mutations, resulting in a neonatal encephalopathy phenotype, MRX phenotype or RTT-like phenotype, depending on the mutation and whether they are hemi- or heterozygous for the mutant allele. It is suggested that about 2% of MR cases are caused by a mutation in the MECP2 gene [Couvert et al., 2001]. As such, screening for MECP2 mutation in nonspecific cases of MR needs to be considered and appropriate genetic counseling is warranted. The X-linked recessive inheritance of MECP2 mutations and the spectrum of phenotypes add to the complex task of elucidating the role of MeCP2 in the nervous system. Why do not MECP2 mutations in MRX demonstrate the same deleterious effect in males as the ones in RTT? How do these mutations relate to the degree of perturbation of MeCP2 function? And how could the spectrum of phenotypes in males and females be explained? These are a few questions that beg further exploration in light of these new findings.

ACKNOWLEDGEMENTS

We would like to thank David Chiu for making Figure 1.

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