

REVIEW ARTICLE

Rett Syndrome and MeCP2: Linking Epigenetics and Neuronal Function

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Introduction

Rett syndrome (RTT [MIM #312750]) was discovered when two girls who exhibited the same unusual behaviors happened to be seated next to each other in the waiting room of Andreas Rett, a Viennese pediatrician. It took >30 years after this discovery to determine the genetic basis of RTT, largely because the disease is primarily sporadic in nature and because familial cases are scarce. The discovery that mutations in methyl-CpG-binding protein 2 (*MECP2*) cause RTT and other neurodevelopmental disorders has called attention to the importance of epigenetic modifications in neuronal function.

Features of RTT

Clinical Features

RTT affects ~1/15,000 females worldwide (Hagberg 1985; Kerr and Stephenson 1985; Kozinetz et al. 1993). The classic form of the disease, found almost exclusively in females, follows a distinct developmental course that Andreas Rett described as “both tragic and fascinating” (Rett 1986). Affected girls are born healthy, appear to develop normally until age 6–18 mo, and achieve the expected motor, language, and social milestones (Hagberg et al. 1983). Their neurological development is then arrested and begins regressing in a predictable pattern comprising roughly four stages (Hagberg and Witt-Engerstrom 1986). During Stage I (age 6–18 mo), girls cease to acquire new skills; they display decelerating head growth and autistic features such as emotional withdrawal and diminished eye contact. In Stage II (age 1–4 years), affected children lose learned skills such as speech and purposeful hand use. They develop irregular breathing patterns, truncal and gait ataxia/apraxia, and

stereotypical hand wringing; about half the girls also develop seizures. There is some stabilization of the disease during Stage III (age 4–7 years), because loss of skills can continue only to a point. During this stage, girls may learn to communicate preferences using eye pointing. They still have gross cognitive and motor impairments and commonly experience epileptic episodes. Seizures become less frequent during Stage IV (age 5–15 years and older), but motor deterioration continues. Hypoactivity, especially among those who cannot walk, contributes to the frequent development of scoliosis, which can cause the girls to be confined to wheelchairs (Hagberg and Witt-Engerstrom 1986).

Other features are associated with RTT but are not diagnostic. For example, patients with RTT are generally small for their age (Holm 1986). Some have suggested that this growth retardation is due to poor self-feeding abilities and reduced caloric intake (Thommessen et al. 1992), but others have found it to be independent of diet (Rice and Haas 1988). Many patients display an abnormally long QT_c interval (Sekul et al. 1994; Guideri et al. 1999). Although females with RTT often live well into adulthood, the mortality rate is 1.2% per year, with about 1/4 of these deaths described as sudden and unexplained (Kerr et al. 1997). Incidents of sudden death have been postulated to result from breathing dysfunction and cardiac abnormalities (Sekul et al. 1994; Guideri et al. 1999). Finally, patients with RTT often have signs of autonomic dysfunction such as constipation and cold or pale extremities (Hagberg et al. 1983; Naidu et al. 1986).

In addition to classic RTT (summarized in table 1), five distinct categories of atypical cases have been delineated on the basis of clinical criteria (Hagberg and Skjeldal 1994). These variants have some, but not all, diagnostic features of RTT and can be milder or more severe. Milder variants include the *forme fruste* (“worn-down form”), the late regression variant, and the preserved speech variant (Hagberg and Witt-Engerstrom 1986; Zappella 1992; Hagberg and Skjeldal 1994). The more severe forms are the congenital form and the early-seizure-onset variant (Hanefeld 1985; Goutieres and Aicardi 1986; Hagberg and Skjeldal 1994).

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Table 1
Phenotypes Associated with *MECP2* Mutations in Human Females

CHARACTERISTIC	PHENOTYPE FOR		
	Classic RTT ^a	Mild RTT Variants ^b	Severe RTT Variants ^c
Age at onset	Onset between 6–18 mo	Later onset	Congenital onset
Head/body size	Small head, body	May have small head, body	Small head, body
Seizures	Seizures		Early seizure onset
Speech	Loss of speech	Speech is preserved	
Motor function	Motor deficiencies	Usually ambulatory	Hypotonia, motor deficiencies
Hand use	Stereotypical hand motions	Retain hand use	
Social Interactions	Autistic features		
Intelligence	Mental retardation	Mild or no mental retardation	Mental retardation
Spinal curvature	Scoliosis and/or kyphosis		Scoliosis and/or kyphosis
Respiration	Breathing dysfunction		Breathing dysfunction

^a Null alleles or severe inactivating mutations, balanced XCI.

^b Hypomorphic alleles (late truncations) with balanced XCI or null alleles with favorably skewed XCI. (Very late truncations and some missense mutations, such as A140V and Q406X, result in no phenotype in females even when XCI is balanced. These same mutations do, however, produce phenotypes in males.)

^c Null alleles or severe inactivating mutations, possibly due to unfavorably skewed XCI.

Neurophysiology

The electroencephalogram (EEG) is typically normal until 3 years of age (Verma et al. 1986; Glaze et al. 1987). After this period, the background activity remains normal, but repetitive high-amplitude spike-and-wave discharges (focal, multifocal, or generalized) are seen. Later, the background activity becomes abnormally slow (Verma et al. 1986). After 10 years of age, the background activity shows additional slowing, but, consistent with the reduction in seizures during the final stage of the disease, the EEG discharges are reduced in frequency, amplitude, and duration (Niedermeyer et al. 1986; Verma et al. 1986).

Breathing appears to be normal during sleep, but periods of irregular breathing (hyperventilation followed by periods of apnea) often occur during wakefulness, particularly when patients are under emotional or physical stress (Lugaresi et al. 1985; Cirignotta et al. 1986; Southall et al. 1988). Because the respiratory abnormalities in RTT are restricted to the wakeful state, it is thought that the voluntary system for regulating breathing is affected rather than the autonomic system, which controls respiration during non-rapid-eye-movement sleep (Lugaresi et al. 1985).

Neuroimaging

Volumetric magnetic resonance imaging studies show an overall decrease in brain volume, affecting gray matter more than white matter, but reveal no evidence of neurodegeneration (Reiss et al. 1993). The caudate nucleus and frontal cortex show the largest reduction in volume (Reiss et al. 1993; Subramaniam et al. 1997). The volume of white matter does increase with age, however, suggesting that myelination progresses normally (Reiss et al. 1993).

As measured by single photon emission-computed tomography, global cerebral blood flow (a measure of neuronal metabolism) is 22% lower in patients with RTT (Nielsen et al. 1990). At a regional level, blood flow in the frontal, parietal, and temporal regions is significantly lower than in control individuals, whereas that in the occipital and sensorimotor areas is normal (Nielsen et al. 1990; Lappalainen et al. 1997). Because the blood flow pattern in the brains of patients with RTT resembles the pattern of normal infants before age 6 mo, it has been proposed that blood flow pattern reflects developmental arrest (Nielsen et al. 1990).

Neuropathology

In spite of a deteriorating clinical course and diverse neurological features, the brain pathology in RTT reveals only subtle abnormalities. As one might expect from both imaging studies and the clinical deceleration in head growth, postmortem studies consistently find that brains of patients with classic RTT weigh 14%–34% less than those of control subjects' brains (Jellinger and Seitelberger 1986). To control for the fact that patients with RTT often have a reduced body size, organ weights have been compared with those of normal individuals of the same height to show that there is a selective decrease in the size of the brain (Armstrong et al. 1999).

RTT was first thought to be a neurodegenerative disease because of the developmental regression, but the majority of neuropathological findings argue against this and indicate that it is a disorder of arrested neuronal development (Armstrong 2001). Impaired neuronal development was initially suggested by the observation that the dendrites of pyramidal neurons of the frontal and motor cortex were reduced in length and complexity

(Armstrong 1992; Armstrong et al. 1995). Three-dimensional confocal microscopy studies confirmed these findings and also revealed a reduction in the number of dendritic spines (Belichenko et al. 1994). Other studies showed that neurons of the cerebral cortex, basal ganglia, thalamus, hippocampus, amygdala, and substantia nigra were smaller and more densely packed than in control subjects (Bauman et al. 1995). The observation of reduced pigmentation in the substantia nigra (Jellinger and Seitelberger 1986) also suggests impaired neuronal development, since melanin pigment normally accumulates in these neurons from the 5th postnatal wk of development until age 12–15 years (Fenichel and Bazelton 1968; Spence and Gilles 1971).

A number of molecular changes are consistent with this neuropathology. The dendritic alterations observed in postmortem tissue could be related to reductions in the levels of microtubule-associated protein 2 (an important cytoskeletal component of neuronal dendrites), substance P (a neuropeptide that has been shown to stimulate neurite extension in cultured neuroblastoma cells), and prostaglandin endoperoxide H synthase-2 (the rate-limiting enzyme in prostanoid biosynthesis that localizes to dendritic spines and marks mature neurons) (Whitty et al. 1993; Kaufmann et al. 1995, 1996, 1997; Matsuishi et al. 1997; Deguchi et al. 2000). These pathological observations support the conclusion that, although glial development occurs normally, neuronal development is hindered. Whether the molecular alterations are a cause or a result of the abnormal development has not been determined.

Genetic Aspects of RTT

Mapping of the Mutated Gene

The mode of inheritance of RTT was difficult to determine, because only ~1% of RTT cases are familial and because affected individuals rarely reproduce (Schanen 1999). Nevertheless, the virtual absence of affected males and the presence of families with affected half-sisters suggested an X-linked dominant inheritance pattern with male lethality (Hagberg et al. 1983; Killian 1986). Consistent with an X-linked mutation, analysis of the available families revealed that inheritance through maternal lineages was common (Zoghbi 1988). Furthermore, in families where the mother was an obligate carrier (e.g., families with affected half-sisters or aunt-niece pairs), X-chromosome inactivation (XCI) studies often revealed skewed XCI patterns in the unaffected mother (Zoghbi et al. 1990; Schanen et al. 1997). Some substantiation for the male-lethality aspect of the X-linked hypothesis was provided by males, born into families with RTT, who displayed neonatal encephalopathy with hypotonia, seizures, apnea, and infantile

death (Ruch et al. 1989; Schanen et al. 1997, 1998). The identification of males who harbored an extra X-chromosome (47,XXY) and bore features identical to those of RTT was also compatible with X-linked inheritance (Vorsanova et al. 1996).

Conventional genomewide linkage analysis was not feasible in RTT because of the rarity of familial cases and the limited vertical transmission. A focused exclusion-mapping approach on the basis of the hypothesis of an X-linked mutation (Archidiacono et al. 1991; Ellison et al. 1992; Curtis et al. 1993; Schanen et al. 1997; Schanen and Francke 1998; Sirianni et al. 1998) culminated in the exclusion of most of the X-chromosome, leaving only the region distal to Xq27.3. Conventional linkage analysis using the few available families with recurrent RTT revealed linkage to markers in this region with a peak LOD score of 2.9 (Sirianni et al. 1998; Webb et al. 1998; Xiang et al. 1998). A number of genes within Xq27.3-qter were analyzed for mutations in patients with RTT because of their known expression or function in the central nervous system (Wan and Francke 1998; Amir et al. 2000a; Xiang et al. 2000), but mutations were finally identified in the methyl-CpG-binding protein 2 gene (*MECP2* [MIM 300005]), a widely expressed transcriptional repressor (Amir et al. 1999). *MECP2* had previously been mapped to human Xq28 and was found to be subject to XCI (Quaderi et al. 1994; Adler et al. 1995; D'Esposito et al. 1996; Vilain et al. 1996).

MECP2 Mutations in Classic RTT

Data from many laboratories have demonstrated that mutations in *MECP2* are the primary cause of RTT, because mutations have been identified in 70%–90% of sporadic cases and ~50% of familial cases (Shahbazian and Zoghbi 2001). Only the coding region has been thoroughly analyzed, however, so mutations in regulatory elements could account for those cases in which no mutation has been identified. Consistent with the prominence of C→T transition mutations at 5'-CG-3' (CpG) sites in other disease genes, presumably resulting from spontaneous deamination of methylated cytosine residues (Cooper and Youssoufian 1988), almost 70% of the reported mutations result from this transition at only eight different CpG dinucleotides within the *MECP2* gene (Lee et al. 2001).

MECP2 Mutations in RTT Variants

As noted above, there are several variants on the classic RTT phenotype, which itself is complex and variable. One of the first questions to be addressed after the discovery of *MECP2* mutations was whether these RTT variants are truly alternate forms of the same entity. Findings indicate that a majority of females with the preserved speech variant do have mutations in *MECP2*

Table 2**Phenotypic Categories for MECP2 Mutations in Human Males**

Allele Type	Phenotype
Null (early truncating mutations and some missense mutations)	(1) Neonatal encephalopathy, severe hypotonia, seizures, apnea, and infantile death; or (2) RTT-like features with 47,XXY karyotype or somatic mosaicism
Hypomorphic (late truncations and some missense mutations)	(1) Mental retardation, no delayed development of language but macrocephaly, tremors, ataxia, hypoactivity, and seizures; (2) childhood-onset schizophrenia with loss of language; or (3) manic-depressive psychosis, pyramidal signs, and macro-orchidism

(De Bona et al. 2000; Yamashita et al. 2001; Zappella et al. 2001). Mutations have also been identified in a fraction (20%–40%) of patients with other forms of the disease, including the *forme fruste* and congenital variants (Buyse et al. 2000; Cheadle et al. 2000; Huppke et al. 2000; Bourdon et al. 2001; Inui et al. 2001; Nielsen et al. 2001) (see table 1). The lower percentage of identified *MECP2* mutations in patients with atypical RTT could indicate the presence of genocopying mutations or could suggest that RTT variants are more frequently associated with (as-yet-unidentified) mutations in regulatory elements of *MECP2* than the classic form of the disease.

Origin of MECP2 mutations

The prediction that most mutations occur de novo (Comings 1986) has been verified with multiple observations that the mutations found in sporadic cases are not present in somatic cells from either parent (Amir et al. 1999; Wan et al. 1999; Bienvenu et al. 2000; Buyse et al. 2000; Erlandson et al. 2001; Monros et al. 2001; Nicolao et al. 2001). Since mutations on the paternal X-chromosome would be inherited by daughters but not sons, some have proposed a high paternal:maternal ratio of de novo mutations to explain the low incidence of affected males (Thomas 1996). This hypothesis is, in fact, correct: haplotype analysis has shown that >95% of *MECP2* mutations originate on the paternal chromosome (Girard et al. 2001; Trappe et al. 2001). A high ratio of male:female germ-cell mutations is not unique to *MECP2*; it has been documented for a number of human disease genes (Crow 2000). It is also known that almost 40% of the mutations responsible for various diseases occur at CpG sites (Cooper and Youssoufian 1988). The higher levels of methylation in male germ cells at early stages of gametogenesis, in addition to the greater number of mitotic divisions in the male germline, could explain sex-specific mutation patterns (Driscoll and Migeon 1990; El-Maarri et al. 1998).

Effect of XCI

Skewed XCI patterns had been postulated to explain the occurrence of unaffected carrier females in families with recurrent RTT (Zoghbi et al. 1990). In support of this idea, skewed XCI (presumably favoring inactivation of the mutant allele) has been observed in females who carry RTT-causing mutations but are asymptomatic or suffer from only mild learning disability (Wan et al. 1999; Amir et al. 2000b; Bienvenu et al. 2000; Villard et al. 2000; Hoffbuhr et al. 2001). Although it seems likely that cells expressing mutant *MECP2* would have some growth disadvantage and be selected against, this appears not to be the case: the same mutation is present in the carrier females and their affected daughters. In fact, the majority of affected females have a balanced pattern of XCI, both in peripheral blood and brain tissue (Amir et al. 2000b; Shahbazian et al. 2002b). Therefore, the skewed XCI pattern in carrier females is probably due to chance.

RTT Mutations in Males

Mutation analysis has also shed light on the question of whether the boys who developed neonatal encephalopathy and died within 1–2 years of birth suffered the consequences of *MECP2* mutations (table 2). Indeed, the same mutations that are present in their sisters with classic RTT proved to be the cause of disease in these boys (Wan et al. 1999; Villard et al. 2000; Hoffbuhr et al. 2001; Geerdink et al. 2002; Zeev et al. 2002). Males bearing a mild *MECP2* mutation that would likely produce a very mild phenotype in girls (i.e., late truncating mutations and some missense mutations) can survive the neonatal period, but they develop severe mental retardation associated with motor abnormalities (table 2). In contrast to these cases, there are several instances of males with RTT-causing *MECP2* mutations that have developed an RTT phenotype. In these cases, some other modifier is present, such as somatic mosaicism for the mutation or a partial or complete Klinefelter (47,XXY) karyotype (Clayton-Smith et al. 2000; Armstrong et al. 2001; Hoffbuhr et al. 2001; Leonard et al. 2001;

Schwartzman et al. 2001; Vorsanova et al. 2001; Topcu et al. 2002). In these situations, the effect of an *MECP2* mutation in a male is mitigated in the same way that it is in a female, that is, by only a fraction of cells expressing the mutant *MECP2* allele.

Genotype-Phenotype Correlation

Several groups have conducted genotype-phenotype correlation studies to determine whether different types of mutations in *MECP2* can account for the variability of clinical features in patients with RTT; these studies have yielded inconsistent results. Two groups found that truncating mutations lead to a more severe overall phenotype than missense mutations (Cheadle et al. 2000; Monros et al. 2001), and one of these studies further showed that late truncations correlate with a less severe outcome than early truncations (Cheadle et al. 2000). In 18 females with the preserved speech variant, all the mutations were either missense or late truncations, further supporting the notion that these types of mutations have milder consequences than the early truncations seen in classic RTT (Zappella et al. 2001). Several other studies, however, have found no significant correlation between the mutation type and the overall severity of clinical features (Amir et al. 2000b; Bienvenu et al. 2000; Huppke et al. 2000; Giunti et al. 2001; Yamada et al. 2001; Chae et al. 2002). This discordance likely results from the fact that the pattern of XCI can influence the phenotypic outcome of mutations in females.

In males, the confounding effect of XCI is absent, and the correlation between the type of mutation and the phenotypic outcome is clearer. As mentioned, *MECP2* mutations that cause classic RTT in females lead to neonatal encephalopathy and infantile death in males unless the mutations are mitigated by somatic mosaicism or a Klinefelter phenotype (table 2). In contrast, other mutations in *MECP2*, which are not known to cause RTT in females, have been found in males manifesting various features, some of which are seen in RTT. For example, a Q406X mutation, which eliminates the last 80 amino acids of the protein, was found in two males with delayed development, macrocephaly, seizures, ataxia, and absence of language (Meloni et al. 2000). Females with this same mutation were unaffected, even with balanced XCI patterns (Meloni et al. 2000).

Many missense mutations have been identified in males with nonspecific X-linked mental retardation (MRX). In fact, the observed frequency of *MECP2* mutations in a large collection of males with MRX (~2%) is almost as high as the frequency of trinucleotide expansions in the *FMR1* gene in this population (~3%–4%) (Couvert et al. 2001). The A140V mutation has been associated with a number of phenotypes: mental retardation; mental retardation with abnormal gait

and speech difficulty; childhood-onset schizophrenia with loss of language; and a syndrome characterized by manic-depressive psychosis, pyramidal signs, and macroorchidism (Orrico et al. 2000; Couvert et al. 2001; Cohen et al. 2002; Klauck et al. 2002). Because males with these mutations survive into adulthood and females with the same mutations are unaffected despite balanced XCI patterns, these types of mutations appear to be less detrimental to MeCP2 function than those causing RTT. Whereas RTT-causing mutations typically lead to either premature truncation or nonconservative amino acid changes, the milder mutations are more often conservative amino acid changes or truncations that preserve a significant portion of the coding region. Given that a single mutation (e.g., A140V) can have disparate effects in different families, other genetic factors probably contribute to this phenotypic variability.

MeCP2 Function

MeCP2 binds preferentially to DNA methylated at CpG sites through an 85–amino acid methyl-CpG-binding domain (MBD) (amino acids 78–162) (Lewis et al. 1992; Nan et al. 1993). Once bound to DNA, MeCP2 is thought to silence transcription of downstream genes by recruiting corepressor complexes through a 104–amino acid transcriptional repression domain (TRD) (amino acids 207–310) (Nan et al. 1997). One complex shown to associate with MeCP2 is the Sin3A corepressor complex (Jones et al. 1998; Nan et al. 1998). This complex contains histone deacetylase (HDAC) 1 and HDAC2 and was originally shown to mediate repression by the DNA-binding heterodimer, Mad-Max (Laherty et al. 1997). MeCP2 also interacts with two other corepressors, the proto-oncoprotein of the Sloan-Kettering virus named after the Sloan-Kettering Institute (c-Ski) and the nuclear receptor corepressor (N-CoR) (Kokura et al. 2001). c-Ski and N-CoR are components of histone deacetylase complexes that can but do not always function together (Heinzel et al. 1997; Nomura et al. 1999). It is interesting that repression by MeCP2 is not completely alleviated by the histone-deacetylase inhibitor, trichostatin A (TSA), suggesting that MeCP2 may also repress transcription in an HDAC-independent manner (Jones et al. 1998; Yu et al. 2000). In support of this idea, the TRD interferes with the assembly of the transcriptional preinitiation complex on naked DNA and interacts directly with transcription factor IIB (TFIIB), a component of the basal transcriptional machinery (Kaludov and Wolffe 2000).

In addition to the MBD and the TRD, there are two other domains of MeCP2 associated with specific functions. First, lying within the TRD is the nuclear localization signal (amino acids 255–271), which is sufficient for transportation of the protein into the nucleus (Nan

et al. 1996). Second, the last 63 amino acids of MeCP2 have been shown to facilitate binding of the protein to both naked and nucleosomal DNA (Chandler et al. 1999). Another study, however, showed that this region decreases binding to methylated DNA but may increase protein stability (Yusufzai and Wolffe 2000).

Effect of Mutations on MeCP2 Function

Missense mutations identified in the *MECP2* gene in patients with RTT cluster in the MBD and TRD, but some are found outside these regions and also in the C-terminus. In vitro studies have demonstrated that many missense mutations within the MBD (R106W, R111G, Y123A, I125A, R133C, F155S, and T158M) significantly reduce the affinity of MeCP2 for methylated DNA (Ballestar et al. 2000; Yusufzai and Wolffe 2000; Free et al. 2001). Consistent with these observations, MeCP2 with the R106W or F155S mutation, when transfected into cells, is impaired in its ability to localize to heterochromatic domains and to repress transcription of a reporter (Kudo et al. 2001). It is interesting that the A140V and E137G mutants (within the MBD), which cause milder phenotypes in humans, localize normally to heterochromatin but have diminished repressive capability (Kudo et al. 2002). This suggests that amino acids in the MBD may have roles outside of binding methyl-CpG sequences or that the heterochromatic localization assay is not sensitive enough to detect the diminished binding affinity. As expected, MeCP2 proteins that retain the MBD but are truncated within the TRD are able to bind methylated DNA but are impaired in their ability to repress transcription (Yusufzai and Wolffe 2000). Less expected is that one missense mutation in the TRD (R306C) does not reduce repressive activity of MeCP2 in a transfection assay, although it may have stronger effects in vivo (Yusufzai and Wolffe 2000). Deletions within the C-terminus of MeCP2, which are common mutations in classic RTT, significantly decrease protein stability (Yusufzai and Wolffe 2000).

DNA Methylation

Because the function of MeCP2 is strongly tied to DNA methylation, an understanding of the role of methylation is necessary to gain insight into the molecular basis of RTT. Whereas promoters of cellular “house-keeping” genes are relatively rich in CpG yet protected from methylation (Bird et al. 1985), other genes are methylated in unique ways. Tissue-specific genes are often methylated in nonexpressing tissues, X-linked genes are methylated on the inactive X-chromosome, and imprinted genes are methylated according to their parental origin. In addition to cellular genes, satellite DNA, retroviruses, and transposable elements are highly methylated (Yoder et al. 1997). Genomewide methylation is

thought to occur early in embryonic development (Lock et al. 1987; Kafri et al. 1992). In the developing germline, methylation patterns are erased, and a second phase of methylation establishes imprinting patterns according to the sex of the embryo (Kafri et al. 1992; Brandeis et al. 1993). DNA methylation is now known to be accomplished by two classes of DNA methyltransferases (DNMTs). DNMT3a and DNMT3b are partially redundant de novo methyltransferases, which establish the methylation pattern (Okano et al. 1998, 1999). DNMT1 is a maintenance methyltransferase, which preserves the methylation pattern through DNA replication by methylating the newly synthesized DNA strand (Bestor and Ingram 1983). Demonstrating the essential role of methylation in gene regulation and development, Dnmt1-deficient mice and mice lacking both Dnmt3a and Dnmt3b die before embryonic day (E) 11.5 (Li et al. 1992; Okano et al. 1999). Because *MECP2* mutations result in a primarily neurological phenotype, it appears that MeCP2 is essential only for the regulation of a subset of methylated genes.

MeCP2 Target Genes

MeCP2 has been shown to bind and/or silence a number of methylated genes, including retroviral, tissue-specific, and imprinted genes. For example, in cell lines infected with the Moloney murine leukemia virus, MeCP2 associates with the provirus in a methylation-dependent manner (Lorincz et al. 2001). LINE-1 but not *Alu* retrotransposons are also repressed by MeCP2 in transfection assays (Yu et al. 2001). Genes methylated in a cell- or tissue-specific manner may also be regulated by MeCP2. For example, the leukosialin gene, which is differentially methylated depending on the tissue, is repressed by MeCP2 in a transfection assay when the promoter is methylated, although whether MeCP2 binds to this promoter in vivo remains to be determined (Kudo 1998). In addition, the multidrug resistance gene promoter in drug-sensitive cells is hypermethylated and bound by MeCP2, whereas in drug-resistant cells, the promoter is hypomethylated, transcriptionally active, and relatively free of MeCP2 (El-Osta and Wolffe 2001; El-Osta et al. 2002). MeCP2 has also been implicated in the regulation of imprinted genes. For example, MeCP2 binds selectively to the paternal allele of the differentially methylated domain of the *H19* gene (Drewell et al. 2002) and to the maternal (methylated) allele of the imprinted *U2af1-rs1* gene (Gregory et al. 2001). It is interesting that several imprinted genes have been implicated in neurological functions, including *Ube3a*, mutated in Angelman syndrome (Kishino et al. 1997; Matsuura et al. 1997), and *Peg1*, an imprinted gene, which, when deleted in mice, causes abnormal maternal behavior and growth retardation (Lefebvre et al. 1998). Whether any

of these genes is abnormally expressed in RTT remains to be determined.

Methyl-CpG-Binding Proteins

Given that methylation is essential for viability in mice, it is not too surprising that a number of proteins other than MeCP2 bind to methylated DNA. A group of proteins, MBD1, MBD2, MBD3, and MBD4, are so named because they contain a methyl-CpG-binding domain similar to that of MeCP2 (Cross et al. 1997; Hendrich and Bird 1998). Another protein, Kaiso, which has homology to the POZ zinc finger family of DNA-binding transcription factors but does not contain the classical MBD motif, has also been shown to specifically bind methylated DNA (Daniel and Reynolds 1999; Prokhortchouk et al. 2001). MBD1, MBD2, and MBD4, like MeCP2, can bind DNA containing only one symmetrically methylated CpG site and can localize to centromeric heterochromatin in transfected cells (Hendrich and Bird 1998; Fujita et al. 1999; Ng et al. 1999). In contrast, Kaiso requires at least two symmetrically methylated CpG sites to bind DNA, and MBD3 lacks the ability to bind methylated DNA in vitro (Hendrich and Bird 1998; Prokhortchouk et al. 2001).

In addition to sharing the ability to bind methylated CpG sites, MBD1, MBD2, and Kaiso are similar to MeCP2 in their ability to repress transcription (Ng et al. 1999; Boeke et al. 2000; Ng et al. 2000; Prokhortchouk et al. 2001). It is interesting to note that Kaiso has no sequence homology to the MBD family members, yet it is able to perform a similar function; thus, the true number of methyl-CpG-binding transcriptional repressors may be much higher than previously suspected. Considering the high level of functional similarity within this group of proteins, it seems likely that there would be at least partial compensation when one member is deleted. The primarily neurological phenotype of *MECP2* mutations, however, leads us to hypothesize that this protein has some function that is specific to neurons and is not compensated for by other members of the MBD family.

MeCP2 Expression Pattern

Studies of the expression pattern of *MECP2* indicate that MeCP2 may indeed have a specialized role in neurons. Analysis of the *MECP2* mRNA has been complicated by the presence of three alternatively spliced transcripts (1.9 kb, 7.5 kb, and 10 kb) produced by differential polyadenylation site usage within the 3'-untranslated region (UTR) (Reichwald et al. 2000). These transcripts are found at varying levels in most tissues examined, with no obvious preference for nervous tissue (D'Esposito et al. 1996; Coy et al. 1999; Reichwald et al. 2000). The apparent tissue-specificity of the three

MECP2 transcripts and the presence of distinct 3'-UTRs suggest that the transcripts might have unique roles. To circumvent the difficulty of interpreting the expression pattern from multiple transcripts, however, the distribution of the MeCP2 protein has been analyzed. Unlike the mRNA, the protein is expressed at higher levels in the brain than in many other tissues (LaSalle et al. 2001; Shahbazian et al. 2002a). Within the brain, MeCP2 is present at high levels in most neurons but not in glia (Akbarian et al. 2001; LaSalle et al. 2001; Shahbazian et al. 2002a).

MeCP2 levels also vary in a neuron-specific manner (LaSalle et al. 2001; Shahbazian et al. 2002a). In the developing cerebral cortex of mouse, human, and non-human primate embryos, the appearance of MeCP2 correlates with neuronal maturation, with earlier-born neurons expressing MeCP2 before later-born neurons (Akbarian et al. 2001; Shahbazian et al. 2002a). These results may partly explain why the brain is most affected in RTT, why the development of white matter progresses normally although neuronal development does not, why certain neuronal populations appear more affected than others, and why the onset of disease is delayed. Understanding the functions of MeCP2 that are specific to mature neurons will be the next challenge in elucidating the pathogenesis of RTT.

Mouse Models of RTT

Tools for studying the in vivo role of MeCP2 have been produced by mutating *Mecp2* in mice (table 3). Male mice with a null mutation in *Mecp2* display stunted body and head growth, hypoactivity, hindlimb-clasping, irregular breathing, and death within 6–10 wk (Chen et al. 2001; Guy et al. 2001) (table 3). Deletion of *Mecp2* in developing neurons results in the same phenotype, demonstrating that the observed deficiencies in the null mice are solely a consequence of neuronal dysfunction and that no overt phenotypes result from MeCP2 dysfunction outside the nervous system, at least within the lifespan of these mice (Guy et al. 2001). These studies also showed that MeCP2 is important not only for developing neurons but also for postmitotic neurons, since deletion of *Mecp2* solely in postmitotic neurons results in a phenotype that is later and milder than that of the null allele (Chen et al. 2001). In *Mecp2*-null mice, concomitant loss of *Mbd2* does not worsen the phenotype, suggesting that *Mbd2* alone does not compensate for the loss of MeCP2 (Guy et al. 2001). Truncation of the C-terminus of MeCP2 in mice leads to a milder phenotype than the null allele, as revealed by the survival of male mice (Shahbazian et al. 2002c). These mice display many features of RTT, including tremors, motor impairments, hypoactivity, anxiety, seizures, kyphosis, and stereotypical forepaw motions (table 3). Elevated levels of acet-

Table 3***Mecp2*-Deficient Mice**

CHARACTERISTIC	MALE MICE		FEMALE MICE WITH HYPOMORPHIC ALLELE ^a
	Null Allele	Hypomorphic Allele	
Age at onset	Appear normal for 5 wk	Appear normal for 6 wk	Appear normal for 6+ wk
Head/body size	Small head, body size		
Involuntary movements	Tremors	Tremors	Tremors
Seizures		Seizures	
Motor function		Motor deficiencies	Motor deficiencies
Motor control	Hindlimb clasping	Stereotypical forepaw motions	Stereotypical forepaw motions
Locomotion	Hypoactivity	Hypoactivity	
Respiration	Irregular breathing		
Spinal curvature		Kyphosis	
Life span	Death at 6–10 wk		
Social interactions		Anxiety and abnormal social interactions	

^a These female mice have not been thoroughly characterized.

ylated histone H3 in brain tissue from these mice suggest that transcriptional alterations may underlie the phenotype (Shahbazian et al. 2002c).

Role of DNA Methylation in Neuronal Function

Because MeCP2 may prove to be important for regulating methylated genes, particularly in neurons, it is worthwhile considering what is known about the role of methylation in neuronal function. It has been questioned for some time why expression levels of the *Dnmt1* maintenance methyltransferase are high in brain, and, more specifically, high in neurons, but are undetectable in glia (Goto et al. 1994). The role of *Dnmt1* in neurons has been studied by conditionally deleting the gene in mice. *Dnmt1* deficiency in neuronal precursors at E9–E10 allows proper development and survival of neurons through birth but adversely affects neuronal survival after birth (Fan et al. 2001). In contrast, deletion of *Dnmt1* in postmitotic neurons does not affect neuronal survival or methylation of retroviral DNA (Fan et al. 2001). The questions that remain are whether *Dnmt1* deficiency in postmitotic neurons leads to changes in the methylation of unique sequences and whether neuronal function is perturbed.

Changes in gene expression mediated by DNA methylation are thought to be important for neuronal maturation. Studies of a model system of neuronal differentiation—PC12 pheochromocytoma cells induced to differentiate with nerve growth factor (NGF)—have shown that gene methylation is required for neurite outgrowth (Persengiev and Kilpatrick 1996). Consistent with this finding, expression levels of several genes are reduced during differentiation of PC12 cells but only in the presence of DNA methyltransferase activity (Persengiev and Kilpatrick 1997). Substantiating the link between neuronal differentiation and chromatin modifications is the observation that the histone deacetylase inhibitor, TSA, also inhibits NGF-induced neurite out-

growth of PC12 cells (Futamura et al. 1995). In vivo evidence supports the role of epigenetic modifications in neuronal development, as several neuronal gene promoters have been found to undergo developmental changes in methylation. For example, the methylation pattern of the neuronal-specific gene *Stac* changes during mouse development (Suzuki et al. 1996). In addition, *GFAP* becomes methylated in neurons but not glia of the rat brain (Barresi et al. 1999). Because both methylation and histone deacetylation appear to be involved in neurite extension of PC12 cells and because dendrites appear to be underdeveloped in RTT brains, it is conceivable that MeCP2 plays a role in mediating the chromatin modifications necessary for dendritic outgrowth.

In addition to changes in neuronal chromatin structure that may occur during development, it is worth considering whether there are changes in chromatin structure in fully differentiated neurons. The fact that *Dnmt3a*^{-/-} mice are normal at birth but show impaired growth and die at 4 wk of age (Okano et al. 1999) suggests that de novo methylation by this enzyme is either important during the embryonic stage for postnatal survival or that it is important after birth. Because *Dnmt3a* is expressed only in a few adult tissues, one of which is the brain (Okano et al. 1998), it is possible that de novo methylation is important in the brain after birth. There is already some evidence to suggest this, since DNA methylation levels increase in neurons after cerebral ischemia induced by temporary blockage of the cerebral artery (Endres et al. 2000). Thus, in postmitotic neurons, neuronal methylation patterns (and, presumably, gene expression) are regulated in response to at least one environmental factor. This leads to the question of whether methylation plays a role in neuronal responses to other types of stress or experiences.

The fact that both *Mbd2* and *Mecp2* mutant mice show primarily or solely neuronal phenotypes that manifest after birth may suggest that these MBD proteins

are specialized to mediate methylation events that occur in mature neurons. *Mecp2* mutant mice display abnormal responses to a novel environment; they are hypoactive and more anxious than wild-type mice, further indicating that methylation might be regulated in response to the environment (Shahbazian et al. 2002c). It is noteworthy that a light pulse applied to mice induces phosphorylation of serine 10 on histone H3, specifically in neurons of the hypothalamic suprachiasmatic nucleus that function as a circadian clock (Crosio et al. 2000). Serine 10 phosphorylation of histone H3 is known to enhance acetylation of the nearby lysine 14 (Cheung et al. 2000). These results suggest that environmental signals can, indeed, dynamically affect chromatin architecture in neurons.

To understand the molecular basis of RTT and the role of epigenetics in neuronal function, it is essential to identify genes that are targets of MeCP2 repression and determine whether their expression is altered in patients or mouse models. Gene-expression analysis of postmortem RTT brain tissue has revealed reduced expression of a number of neuronal-specific genes and upregulation of some glial-specific genes, although these changes likely reflect secondary changes that occur long after the initial insult (Colantuoni et al. 2001). Now that mouse models of RTT are available, it will be easier to dissect the primary changes in gene expression and neuronal function that underlie the RTT phenotype.

Concluding Remarks

It is becoming evident that MeCP2, a protein originally thought of as a global transcriptional repressor, is actually specialized for a function in neurons of the central nervous system. It is remarkable that mouse models have reproduced virtually every aspect of RTT, including the highly specialized hand-wringing behaviors, suggesting that the pathways leading from dysfunctional MeCP2 to each of these features are conserved between humans and mice. Gene expression analyses in these mouse models may help elucidate the neuronal-specific functions of MeCP2 that are misregulated in RTT. To understand the basis for the selective function of MeCP2, it may be worthwhile to search for additional interacting factors. Given that, in humans, the phenotypic outcome of *MECP2* truncation mutations depends on the position of the truncation, different regions of MeCP2 may interact with particular proteins or complexes. Uncovering the molecular alterations that lead to RTT will not only provide insight into the disease pathogenesis but may also shed light on the normal role of epigenetic modifications in the function of developing and mature neurons.

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for RTT [MIM 312750] and MeCP2 [MIM 300005])

References

- Adler DA, Quaderi NA, Brown SD, Chapman VM, Moore J, Tate P, Distechi CM (1995) The X-linked methylated DNA binding protein, *Mecp2*, is subject to X inactivation in the mouse. *Mamm Genome* 6:491–492
- Akbarian S, Chen RZ, Gribnau J, Rasmussen TP, Fong H, Jaenisch R, Jones EG (2001) Expression pattern of the Rett syndrome gene MeCP2 in primate prefrontal cortex. *Neurobiol Dis* 8:784–791
- Amir R, Dahle EJ, Toriolo D, Zoghbi HY (2000a) Candidate gene analysis in Rett syndrome and the identification of 21 SNPs in Xq. *Am J Med Genet* 90:69–71
- Amir RE, Van den Veyver IB, Schultz R, Malicki DM, Tran CQ, Dahle EJ, Philippi A, Timar L, Percy AK, Motil KJ, Lichtarge O, Smith EO, Glaze DG, Zoghbi HY (2000b) Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes. *Ann Neurol* 47:670–679
- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY (1999) Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2. *Nat Genet* 23:185–188
- Archidiacono N, Lerone M, Rocchi M, Anvret M, Ozcelik T, Francke U, Romeo G (1991) Rett syndrome: exclusion mapping following the hypothesis of germinal mosaicism for new X-linked mutations. *Hum Genet* 86:604–606
- Armstrong D, Dunn JK, Antalffy B, Trivedi R (1995) Selective dendritic alterations in the cortex of Rett syndrome. *J Neuropathol Exp Neurol* 54:195–201
- Armstrong DD (1992) The neuropathology of the Rett syndrome. *Brain Dev* 14 Suppl:S89–S98
- (2001) Rett syndrome neuropathology review 2000. *Brain Dev* 23 Suppl 1:S72–S76
- Armstrong DD, Dunn JK, Schultz RJ, Herbert DA, Glaze DG, Motil KJ (1999) Organ growth in Rett syndrome: a post-mortem examination analysis. *Pediatr Neurol* 20:125–129
- Armstrong J, Pineda M, Aibar E, Gean E, Monros E (2001) Classic Rett syndrome in a boy as a result of somatic mosaicism for a *MECP2* mutation. *Ann Neurol* 50:692
- Ballestar E, Yusufzai TM, Wolffe AP (2000) Effects of Rett syndrome mutations of the methyl-CpG binding domain of the transcriptional repressor MeCP2 on selectivity for association with methylated DNA. *Biochemistry* 39:7100–7106
- Barresi V, Condorelli DF, Giuffrida Stella AM (1999) GFAP gene methylation in different neural cell types from rat brain. *Int J Dev Neurosci* 17:821–828
- Bauman ML, Kemper TL, Arin DM (1995) Pervasive neuroanatomic abnormalities of the brain in three cases of Rett's syndrome. *Neurology* 45:1581–1586
- Belichenko PV, Oldfors A, Hagberg B, Dahlstrom A (1994)

- Rett syndrome: 3-D confocal microscopy of cortical pyramidal dendrites and afferents. *Neuroreport* 5:1509–1513
- Bestor TH, Ingram VM (1983) Two DNA methyltransferases from murine erythroleukemia cells: purification, sequence specificity, and mode of interaction with DNA. *Proc Natl Acad Sci USA* 80:5559–5563
- Bienvenu T, Carrie A, de Roux N, Vinet MC, Jonveaux P, Couvert P, Villard L, Arzimanoglou A, Beldjord C, Fontes M, Tardieu M, Chelly J (2000) MECP2 mutations account for most cases of typical forms of Rett syndrome. *Hum Mol Genet* 9:1377–1384
- Bird A, Taggart M, Frommer M, Miller OJ, Macleod D (1985) A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. *Cell* 40:91–99
- Boeke J, Ammerpohl O, Kegel S, Moehren U, Renkawitz R (2000) The minimal repression domain of MBD2b overlaps with the methyl-CpG-binding domain and binds directly to Sin3A. *J Biol Chem* 275:34963–34967
- Bourdon V, Philippe C, Labrune O, Amsellem D, Arnould C, Jonveaux P (2001) A detailed analysis of the MECP2 gene: prevalence of recurrent mutations and gross DNA rearrangements in Rett syndrome patients. *Hum Genet* 108:43–50
- Brandeis M, Kafri T, Ariel M, Chaillet JR, McCarrey J, Razin A, Cedar H (1993) The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. *Embo J* 12:3669–3677
- Buyse IM, Fang P, Hoon KT, Amir RE, Zoghbi HY, Roa BB (2000) Diagnostic testing for Rett syndrome by DHPLC and direct sequencing analysis of the *MECP2* gene: identification of several novel mutations and polymorphisms. *Am J Hum Genet* 67:1428–1436
- Chae JH, Hwang YS, Kim KJ (2002) Mutation analysis of MECP2 and clinical characterization in Korean patients with Rett syndrome. *J Child Neurol* 17:33–36
- Chandler SP, Guschin D, Landsberger N, Wolffe AP (1999) The methyl-CpG binding transcriptional repressor MeCP2 stably associates with nucleosomal DNA. *Biochemistry* 38:7008–7018
- Cheadle JP, Gill H, Fleming N, Maynard J, Kerr A, Leonard H, Krawczak M, Cooper DN, Lynch S, Thomas N, Hughes H, Hulten M, Ravine D, Sampson JR, Clarke A (2000) Long-read sequence analysis of the MECP2 gene in Rett syndrome patients: correlation of disease severity with mutation type and location. *Hum Mol Genet* 9:1119–1129
- Chen RZ, Akbarian S, Tudor M, Jaenisch R (2001) Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat Genet* 27:327–331
- Cheung P, Tanner KG, Cheung WL, Sassone-Corsi P, Denu JM, Allis CD (2000) Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol Cell* 5:905–915
- Cirignotta F, Lugaresi E, Montagna P (1986) Breathing impairment in Rett syndrome. *Am J Med Genet Suppl* 1:167–173
- Clayton-Smith J, Watson P, Ramsden S, Black GC (2000) Somatic mutation in MECP2 as a non-fatal neurodevelopmental disorder in males. *Lancet* 356:830–832
- Cohen D, Lazar G, Couvert P, Desportes V, Lippe D, Mazet P, Heron D (2002) MECP2 mutation in a boy with language disorder and schizophrenia. *Am J Psychiatry* 159:148–149
- Colantuoni C, Jeon OH, Hyder K, Chenchik A, Khimani AH, Narayanan V, Hoffman EP, Kaufmann WE, Naidu S, Pevsner J (2001) Gene expression profiling in postmortem Rett syndrome brain: differential gene expression and patient classification. *Neurobiol Dis* 8:847–865
- Comings DE (1986) The genetics of Rett syndrome: the consequences of a disorder where every case is a new mutation. *Am J Med Genet Suppl* 1:383–388
- Cooper DN, Youssoufian H (1988) The CpG dinucleotide and human genetic disease. *Hum Genet* 78:151–155
- Couvert P, Bienvenu T, Aquaviva C, Poirier K, Moraine C, Gendrot C, Verloes A, Andres C, Le Fevre AC, Souville I, Steffann J, des Portes V, Ropers HH, Yntema HG, Fryns JP, Briault S, Chelly J, Cherif B (2001) MECP2 is highly mutated in X-linked mental retardation. *Hum Mol Genet* 10:941–946
- Coy JF, Sedlacek Z, Bachner D, Delius H, Poustka A (1999) A complex pattern of evolutionary conservation and alternative polyadenylation within the long 3'-untranslated region of the methyl-CpG-binding protein 2 gene (MeCP2) suggests a regulatory role in gene expression. *Hum Mol Genet* 8:1253–1262
- Crosio C, Cermakian N, Allis CD, Sassone-Corsi P (2000) Light induces chromatin modification in cells of the mammalian circadian clock. *Nat Neurosci* 3:1241–1247
- Cross SH, Meehan RR, Nan X, Bird A (1997) A component of the transcriptional repressor MeCP1 shares a motif with DNA methyltransferase and HRX proteins. *Nat Genet* 16:256–259
- Crow JF (2000) The origins, patterns and implications of human spontaneous mutation. *Nat Rev Genet* 1:40–47
- Curtis AR, Headland S, Lindsay S, Thomas NS, Boye E, Kamakari S, Roustan P, Anvret M, Wahlstrom J, McCarthy G (1993) X chromosome linkage studies in familial Rett syndrome. *Hum Genet* 90:551–555
- Daniel JM, Reynolds AB (1999) The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. *Mol Cell Biol* 19:3614–3623
- De Bona C, Zappella M, Hayek G, Meloni I, Vitelli F, Bruttini M, Cusano R, Loffredo P, Longo I, Renieri A (2000) Preserved speech variant is allelic of classic Rett syndrome. *Eur J Hum Genet* 8:325–330
- Deguchi K, Antalffy BA, Twohill LJ, Chakraborty S, Glaze DG, Armstrong DD (2000) Substance P immunoreactivity in Rett syndrome. *Pediatr Neurol* 22:259–266
- D'Esposito M, Quaderi NA, Ciccociola A, Bruni P, Esposito T, D'Urso M, Brown SD (1996) Isolation, physical mapping, and northern analysis of the X-linked human gene encoding methyl CpG-binding protein, MECP2. *Mamm Genome* 7:533–535
- Drewell RA, Goddard CJ, Thomas JO, Surani MA (2002) Methylation-dependent silencing at the H19 imprinting control region by MeCP2. *Nucleic Acids Res* 30:1139–1144
- Driscoll DJ, Migeon BR (1990) Sex difference in methylation of single-copy genes in human meiotic germ cells: implications for X chromosome inactivation, parental imprinting, and origin of CpG mutations. *Somat Cell Mol Genet* 16:267–282

- El-Maarri O, Olek A, Balaban B, Montag M, van der Ven H, Urman B, Olek K, Caglayan SH, Walter J, Oldenburg J (1998) Methylation levels at selected CpG sites in the factor VIII and FGFR3 genes, in mature female and male germ cells: implications for male-driven evolution. *Am J Hum Genet* 63:1001–1008
- El-Osta A, Kantharidis P, Zalberg JR, Wolffe AP (2002) Precipitous release of methyl-CpG binding protein 2 and histone deacetylase 1 from the methylated human multidrug resistance gene (MDR1) on activation. *Mol Cell Biol* 22:1844–1857
- El-Osta A, Wolffe AP (2001) Analysis of chromatin-immunopurified MeCP2-associated fragments. *Biochem Biophys Res Commun* 289:733–737
- Ellison KA, Fill CP, Terwilliger J, DeGennaro LJ, Martin-Gallardo A, Anvret M, Percy AK, Ott J, Zoghbi H (1992) Examination of X chromosome markers in Rett syndrome: exclusion mapping with a novel variation on multilocus linkage analysis. *Am J Hum Genet* 50:278–287
- Endres M, Meisel A, Biniszkiwicz D, Namura S, Prass K, Ruscher K, Lipski A, Jaenisch R, Moskowitz MA, Dirnagl U (2000) DNA methyltransferase contributes to delayed ischemic brain injury. *J Neurosci* 20:3175–3181
- Erlanson A, Hallberg B, Hagberg B, Wahlstrom J, Martinsson T (2001) MECP2 mutation screening in Swedish classical Rett syndrome females. *Eur Child Adolesc Psychiatry* 10:117–121
- Fan G, Beard C, Chen RZ, Csankovszki G, Sun Y, Siniatia M, Biniszkiwicz D, Bates B, Lee PP, Kuhn R, Trumpp A, Poon C, Wilson CB, Jaenisch R (2001) DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *J Neurosci* 21:788–797
- Fenichel GM, Bazelon M (1968) Studies on neuromelanin. II. Melanin in the brainstems of infants and children. *Neurology* 18:817–820
- Free A, Wakefield RI, Smith BO, Dryden DT, Barlow PN, Bird AP (2001) DNA recognition by the methyl-CpG binding domain of MeCP2. *J Biol Chem* 276:3353–3360
- Fujita N, Takebayashi S, Okumura K, Kudo S, Chiba T, Saya H, Nakao M (1999) Methylation-mediated transcriptional silencing in euchromatin by methyl-CpG binding protein MBD1 isoforms. *Mol Cell Biol* 19:6415–6426
- Futamura M, Monden Y, Okabe T, Fujita-Yoshigaki J, Yokoyama S, Nishimura S (1995) Trichostatin A inhibits both ras-induced neurite outgrowth of PC12 cells and morphological transformation of NIH3T3 cells. *Oncogene* 10:1119–1123
- Geerdink N, Rotteveel JJ, Lammens M, Sistermans EA, Heikens GT, Gabreels FJ, Mullaart RA, Hamel BC (2002) MECP2 mutation in a boy with severe neonatal encephalopathy: clinical, neuropathological and molecular findings. *Neuropediatrics* 33:33–36
- Girard M, Couvert P, Carrie A, Tardieu M, Chelly J, Beldjoud C, Bienvenu T (2001) Parental origin of de novo MECP2 mutations in Rett syndrome. *Eur J Hum Genet* 9:231–236
- Giunti L, Pelagatti S, Lazzarini V, Guarducci S, Lapi E, Coviello S, Ceconi A, Ombroni L, Andreucci E, Sani I, Brusaferrari A, Lasagni A, Ricotti G, Giometto B, Nicolao P, Gasparini P, Granatiero M, Uzielli ML (2001) Spectrum and distribution of MECP2 mutations in 64 Italian Rett syndrome girls: tentative genotype/phenotype correlation. *Brain Dev* 23 Suppl 1:S242–S245
- Glaze DG, Frost JD Jr., Zoghbi HY, Percy AK (1987) Rett's syndrome: correlation of electroencephalographic characteristics with clinical staging. *Arch Neurol* 44:1053–1056
- Goto K, Numata M, Komura JI, Ono T, Bestor TH, Kondo H (1994) Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* 56:39–44
- Goutieres F, Aicardi J (1986) Atypical forms of Rett syndrome. *Am J Med Genet Suppl* 1:183–194
- Gregory RI, Randall TE, Johnson CA, Khosla S, Hatada I, O'Neill LP, Turner BM, Feil R (2001) DNA methylation is linked to deacetylation of histone H3, but not H4, on the imprinted genes *Snrpn* and *U2af1-rs1*. *Mol Cell Biol* 21:5426–5436
- Guideri F, Acampa M, Hayek G, Zappella M, Di Perri T (1999) Reduced heart rate variability in patients affected with Rett syndrome: a possible explanation for sudden death. *Neuropediatrics* 30:146–148
- Guy J, Hendrich B, Holmes M, Martin JE, Bird A (2001) A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat Genet* 27:322–326
- Hagberg B (1985) Rett's syndrome: prevalence and impact on progressive severe mental retardation in girls. *Acta Paediatr Scand* 74:405–408
- Hagberg B, Aicardi J, Dias K, Ramos O (1983) A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Ann Neurol* 14:471–479
- Hagberg B, Witt-Engerstrom I (1986) Rett syndrome: a suggested staging system for describing impairment profile with increasing age towards adolescence. *Am J Med Genet Suppl* 1:47–59
- Hagberg BA, Skjeldal OH (1994) Rett variants: a suggested model for inclusion criteria. *Pediatr Neurol* 11:5–11
- Hanefeld F (1985) The clinical pattern of the Rett syndrome. *Brain Dev* 7:320–325
- Heinzel T, Lavinsky RM, Mullen TM, Soderstrom M, Laherty CD, Torchia J, Yang WM, Brard G, Ngo SD, Davie JR, Seto E, Eisenman RN, Rose DW, Glass CK, Rosenfeld MG (1997) A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* 387:43–48
- Hendrich B, Bird A (1998) Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol* 18:6538–6547
- Hoffbuhr K, Devaney JM, LaFleur B, Sirianni N, Scacheri C, Giron J, Schuette J, Innis J, Marino M, Philippart M, Narayanan V, Umansky R, Kronn D, Hoffman EP, Naidu S (2001) MeCP2 mutations in children with and without the phenotype of Rett syndrome. *Neurology* 56:1486–1495
- Holm VA (1986) Physical growth and development in patients with Rett syndrome. *Am J Med Genet Suppl* 1:119–126
- Huppke P, Laccone F, Kramer N, Engel W, Hanefeld F (2000) Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. *Hum Mol Genet* 9:1369–1375
- Inui K, Akagi M, Ono J, Tsukamoto H, Shimono K, Mano T, Imai K, Yamada M, Muramatsu T, Sakai N, Okada S (2001)

- Mutational analysis of MECP2 in Japanese patients with atypical Rett syndrome. *Brain Dev* 23:212–215
- Jellinger K, Seitelberger F (1986) Neuropathology of Rett syndrome. *Am J Med Genet Suppl* 1:259–288
- Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* 19:187–191
- Kafri T, Ariel M, Brandeis M, Shemer R, Urven L, McCarrey J, Cedar H, Razin A (1992) Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. *Genes Dev* 6:705–714
- Kaludov NK, Wolffe AP (2000) MeCP2 driven transcriptional repression in vitro: selectivity for methylated DNA, action at a distance and contacts with the basal transcription machinery. *Nucleic Acids Res* 28:1921–1928
- Kaufmann WE, Naidu S, Budden S (1995) Abnormal expression of microtubule-associated protein 2 (MAP-2) in neocortex in Rett syndrome. *Neuropediatrics* 26:109–113
- Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P (1996) COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. *Proc Natl Acad Sci USA* 93:2317–2321
- Kaufmann WE, Worley PF, Taylor CV, Bremer M, Isakson PC (1997) Cyclooxygenase-2 expression during rat neocortical development and in Rett syndrome. *Brain Dev* 19:25–34
- Kerr AM, Armstrong DD, Prescott RJ, Doyle D, Kearney DL (1997) Rett syndrome: analysis of deaths in the British survey. *Eur Child Adolesc Psychiatry* 6:71–74
- Kerr AM, Stephenson JB (1985) Rett's syndrome in the west of Scotland. *Br Med J (Clin Res Ed)* 291:579–582
- Killian W (1986) On the genetics of Rett syndrome: analysis of family and pedigree data. *Am J Med Genet Suppl* 1:369–376
- Kishino T, Lalonde M, Wagstaff J (1997) UBE3A/E6-AP mutations cause Angelman syndrome. *Nat Genet* 15:70–73
- Klauck SM, Lindsay S, Beyer KS, Splitt M, Burn J, Poustka A (2002) A mutation hot spot for nonspecific X-linked mental retardation in the *MECP2* gene causes the PPM-X syndrome. *Am J Hum Genet* 70:1034–1037
- Kokura K, Kaul SC, Wadhwa R, Nomura T, Khan MM, Shinagawa T, Yasukawa T, Colmenares C, Ishii S (2001) The Ski protein family is required for MeCP2-mediated transcriptional repression. *J Biol Chem* 276:34115–34121
- Kozinetz CA, Skender ML, MacNaughton N, Almes MJ, Schultz RJ, Percy AK, Glaze DG (1993) Epidemiology of Rett syndrome: a population-based registry. *Pediatrics* 91:445–450
- Kudo S (1998) Methyl-CpG-binding protein MeCP2 represses Sp1-activated transcription of the human leukosialin gene when the promoter is methylated. *Mol Cell Biol* 18:5492–5499
- Kudo S, Nomura Y, Segawa M, Fujita N, Nakao M, Dragich J, Schanen C, Tamura M (2001) Functional analyses of MeCP2 mutations associated with Rett syndrome using transient expression systems. *Brain Dev* 23 Suppl 1:S165–S173
- Kudo S, Nomura Y, Segawa M, Fujita N, Nakao M, Hammer S, Schanen C, Terai I, Tamura M (2002) Functional characterization of MeCP2 mutations found in male patients with X linked mental retardation. *J Med Genet* 39:132–136
- Laherty CD, Yang WM, Sun JM, Davie JR, Seto E, Eisenman RN (1997) Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell* 89:349–356
- Lappalainen R, Liewendahl K, Sainio K, Nikkinen P, Riikonen RS (1997) Brain perfusion SPECT and EEG findings in Rett syndrome. *Acta Neurol Scand* 95:44–50
- LaSalle JM, Goldstine J, Balmer D, Greco CM (2001) Quantitative localization of heterogeneous methyl-CpG-binding protein 2 (MeCP2) expression phenotypes in normal and Rett syndrome brain by laser scanning cytometry. *Hum Mol Genet* 10:1729–1740
- Lee SS, Wan M, Francke U (2001) Spectrum of MECP2 mutations in Rett syndrome. *Brain Dev* 23 Suppl 1:S138–S143
- Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA (1998) Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene *Mest*. *Nat Genet* 20:163–169
- Leonard H, Silberstein J, Falk R, Houwink-Manville I, Ellaway C, Raffaele LS, Engerstrom IW, Schanen C (2001) Occurrence of Rett syndrome in boys. *J Child Neurol* 16:333–338
- Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* 69:905–914
- Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69:915–926
- Lock LF, Takagi N, Martin GR (1987) Methylation of the *Hprt* gene on the inactive X occurs after chromosome inactivation. *Cell* 48:39–46
- Lorincz MC, Schubeler D, Groudine M (2001) Methylation-mediated proviral silencing is associated with *mecp2* recruitment and localized histone H3 deacetylation. *Mol Cell Biol* 21:7913–7922
- Lugaresi E, Cirignotta F, Montagna P (1985) Abnormal breathing in the Rett syndrome. *Brain Dev* 7:329–333
- Matsuishi T, Nagamitsu S, Yamashita Y, Murakami Y, Kimura A, Sakai T, Shoji H, Kato H, Percy AK (1997) Decreased cerebrospinal fluid levels of substance P in patients with Rett syndrome. *Ann Neurol* 42:978–981
- Matsuura T, Sutcliffe JS, Fang P, Galjaard RJ, Jiang YH, Benton CS, Rommens JM, Beaudet AL (1997) De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (*UBE3A*) in Angelman syndrome. *Nat Genet* 15:74–77
- Meloni I, Bruttini M, Longo I, Mari F, Rizzolio F, D'Adamo P, Denvriendt K, Fryns JP, Toniolo D, Renieri A (2000) A mutation in the Rett syndrome gene, *MECP2*, causes X-linked mental retardation and progressive spasticity in males. *Am J Hum Genet* 67:982–985
- Monros E, Armstrong J, Aibar E, Poo P, Canos I, Pineda M (2001) Rett syndrome in Spain: mutation analysis and clinical correlations. *Brain Dev* 23 Suppl 1:S251–S253
- Naidu S, Murphy M, Moser HW, Rett A (1986) Rett syndrome—natural history in 70 cases. *Am J Med Genet Suppl* 1:61–72
- Nan X, Campoy FJ, Bird A (1997) MeCP2 is a transcriptional

- repressor with abundant binding sites in genomic chromatin. *Cell* 88:471–481
- Nan X, Meehan RR, Bird A (1993) Dissection of the methyl-CpG binding domain from the chromosomal protein MeCP2. *Nucleic Acids Res* 21:4886–4892
- Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 393:386–389
- Nan X, Tate P, Li E, Bird A (1996) DNA methylation specifies chromosomal localization of MeCP2. *Mol Cell Biol* 16:414–421
- Ng HH, Jeppesen P, Bird A (2000) Active repression of methylated genes by the chromosomal protein MBD1. *Mol Cell Biol* 20:1394–1406
- Ng HH, Zhang Y, Hendrich B, Johnson CA, Turner BM, Erdjument-Bromage H, Tempst P, Reinberg D, Bird A (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat Genet* 23:58–61
- Nicolao P, Carella M, Giometto B, Tavolato B, Cattin R, Giovannucci-Uzielli ML, Vacca M, Regione FD, Piva S, Bortoluzzi S, Gasparini P (2001) DHPLC analysis of the MECP2 gene in Italian Rett patients. *Hum Mutat* 18:132–140
- Niedermeyer E, Rett A, Renner H, Murphy M, Naidu S (1986) Rett syndrome and the electroencephalogram. *Am J Med Genet Suppl* 1:195–199
- Nielsen JB, Friberg L, Lou H, Lassen NA, Sam IL (1990) Immature pattern of brain activity in Rett syndrome. *Arch Neurol* 47:982–986
- Nielsen JB, Henriksen KF, Hansen C, Silahatoglu A, Schwartz M, Tommerup N (2001) MECP2 mutations in Danish patients with Rett syndrome: high frequency of mutations but no consistent correlations with clinical severity or with the X chromosome inactivation pattern. *Eur J Hum Genet* 9:178–184
- Nomura T, Khan MM, Kaul SC, Dong HD, Wadhwa R, Colmenares C, Kohno I, Ishii S (1999) Ski is a component of the histone deacetylase complex required for transcriptional repression by Mad and thyroid hormone receptor. *Genes Dev* 13:412–423
- Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99:247–257
- Okano M, Xie S, Li E (1998) Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 19:219–220
- Orrico A, Lam C, Galli L, Dotti MT, Hayek G, Tong SF, Poon PM, Zappella M, Federico A, Sorrentino V (2000) MECP2 mutation in male patients with non-specific X-linked mental retardation. *FEBS Lett* 481:285–288
- Persengiev SP, Kilpatrick DL (1997) The DNA methyltransferase inhibitor 5-azacytidine specifically alters the expression of helix-loop-helix proteins Id1, Id2 and Id3 during neuronal differentiation. *Neuroreport* 8:2091–2095
- Persengiev SP, Kilpatrick DL (1996) Nerve growth factor induced differentiation of neuronal cells requires gene methylation. *Neuroreport* 8:227–231
- Prokhortchouk A, Hendrich B, Jorgensen H, Ruzov A, Wilm M, Georgiev G, Bird A, Prokhortchouk E (2001) The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev* 15:1613–1618
- Quaderi NA, Meehan RR, Tate PH, Cross SH, Bird AP, Chatterjee A, Herman GE, Brown SD (1994) Genetic and physical mapping of a gene encoding a methyl CpG binding protein, Mecp2, to the mouse X chromosome. *Genomics* 22:648–651
- Reichwald K, Thiesen J, Wiehe T, Weitzel J, Poustka WA, Rosenthal A, Platzer M, Stratling WH, Kioschis P (2000) Comparative sequence analysis of the MECP2-locus in human and mouse reveals new transcribed regions. *Mamm Genome* 11:182–190
- Reiss AL, Faruque F, Naidu S, Abrams M, Beaty T, Bryan RN, Moser H (1993) Neuroanatomy of Rett syndrome: a volumetric imaging study. *Ann Neurol* 34:227–234
- Rett A (1986) Rett syndrome: history and general overview. *Am J Med Genet Suppl* 1:21–25
- Rice MA, Haas RH (1988) The nutritional aspects of Rett syndrome. *J Child Neurol* 3:S35–S42
- Ruch A, Kurczynski TW, Velasco ME (1989) Mitochondrial alterations in Rett syndrome. *Pediatr Neurol* 5:320–323
- Schanen C, Francke U (1998) A severely affected male born into a Rett syndrome kindred supports X-linked inheritance and allows extension of the exclusion map. *Am J Hum Genet* 63:267–269
- Schanen NC (1999) Molecular approaches to the Rett syndrome gene. *J Child Neurol* 14:806–814
- Schanen NC, Dahle EJ, Capozzoli F, Holm VA, Zoghbi HY, Francke U (1997) A new Rett syndrome family consistent with X-linked inheritance expands the X chromosome exclusion map. *Am J Hum Genet* 61:634–641
- Schanen NC, Kurczynski TW, Brunelle D, Woodcock MM, Dure LS, Percy AK (1998) Neonatal encephalopathy in two boys in families with recurrent Rett syndrome. *J Child Neurol* 13:229–231
- Schwartzman JS, Bernardino A, Nishimura A, Gomes RR, Zatz M (2001) Rett syndrome in a boy with a 47,XXY karyotype confirmed by a rare mutation in the MECP2 gene. *Neuropediatrics* 32:162–164
- Sekul EA, Moak JP, Schultz RJ, Glaze DG, Dunn JK, Percy AK (1994) Electrocardiographic findings in Rett syndrome: an explanation for sudden death? *J Pediatr* 125:80–82
- Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY (2002a) Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Hum Mol Genet* 11:115–124
- Shahbazian MD, Sun Y, Zoghbi HY (2002b) Balanced X chromosome inactivation patterns in the Rett syndrome brain. *Am J Med Genet* 111:164–168
- Shahbazian MD, Young JI, Yuva-Paylor LA, Spencer CM, Antalffy BA, Noebels JL, Armstrong DL, Paylor R, Zoghbi HY (2002c) Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron* 35:243–254
- Shahbazian MD, Zoghbi HY (2001) Molecular genetics of Rett syndrome and clinical spectrum of MECP2 mutations. *Curr Opin Neurol* 14:171–176
- Sirianni N, Naidu S, Pereira J, Pillotto RF, Hoffman EP (1998) Rett syndrome: confirmation of X-linked dominant inheri-

- tance, and localization of the gene to Xq28. *Am J Hum Genet* 63:1552–1558
- Southall DP, Kerr AM, Tirosh E, Amos P, Lang MH, Stephenson JB (1988) Hyperventilation in the awake state: potentially treatable component of Rett syndrome. *Arch Dis Child* 63:1039–1048
- Spence AM, Gilles FH (1971) Underpigmentation of the substantia nigra in chronic disease in children. *Neurology* 21:386–390
- Subramaniam B, Naidu S, Reiss AL (1997) Neuroanatomy in Rett syndrome: cerebral cortex and posterior fossa. *Neurology* 48:399–407
- Suzuki H, Kawai J, Taga C, Yaoi T, Hara A, Hirose K, Hayashizaki Y, Watanabe S (1996) Stac, a novel neuron-specific protein with cysteine-rich and SH3 domains. *Biochem Biophys Res Commun* 229:902–909
- Thomas GH (1996) High male:female ratio of germ-line mutations: an alternative explanation for postulated gestational lethality in males in X-linked dominant disorders. *Am J Hum Genet* 58:1364–1368
- Thomassen M, Kase BF, Heiberg A (1992) Growth and nutrition in 10 girls with Rett syndrome. *Acta Paediatr* 81:686–690
- Topcu M, Akyerli C, Sayi A, Toruner GA, Kocoglu SR, Cimbiş M, Ozcelik T (2002) Somatic mosaicism for a MECP2 mutation associated with classic Rett syndrome in a boy. *Eur J Hum Genet* 10:77–81
- Trappe R, Laccone F, Cobilanschi J, Meins M, Huppke P, Hanefeld F, Engel W (2001) MECP2 mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. *Am J Hum Genet* 68:1093–1101
- Verma NP, Chheda RL, Nigro MA, Hart ZH (1986) Electroencephalographic findings in Rett syndrome. *Electroencephalogr Clin Neurophysiol* 64:394–401
- Vilain A, Apiou F, Vogt N, Dutrillaux B, Malfroy B (1996) Assignment of the gene for methyl-CpG-binding protein 2 (MECP2) to human chromosome band Xq28 by in situ hybridization. *Cytogenet Cell Genet* 74:293–294
- Villard L, Cardoso AK, Chelly PJ, Tardieu PM, Fontes M (2000) Two affected boys in a Rett syndrome family: clinical and molecular findings. *Neurology* 55:1188–1193
- Vorsanova SG, Demidova IA, Ulas V, Soloviev IV, Kazantzeva LZ, Yurov YB (1996) Cytogenetic and molecular-cytogenetic investigation of Rett syndrome: analysis of 31 cases. *Neuroreport* 8:187–189
- Vorsanova SG, Yurov YB, Ulas VY, Demidova IA, Sharonin VO, Kolotii AD, Gorbachevskaya NL, Beresheva AK, Soloviev IV (2001) Cytogenetic and molecular-cytogenetic studies of Rett syndrome (RTT): a retrospective analysis of a Russian cohort of RTT patients (the investigation of 57 girls and three boys). *Brain Dev* 23 Suppl 1:S196–S201
- Wan M, Francke U (1998) Evaluation of two X chromosomal candidate genes for Rett syndrome: glutamate dehydrogenase-2 (GLUD2) and rab GDP-dissociation inhibitor (GDI1). *Am J Med Genet* 78:169–172
- Wan M, Lee SS, Zhang X, Houwink-Manville I, Song HR, Amir RE, Budden S, Naidu S, Pereira JL, Lo IF, Zoghbi HY, Schanen NC, Francke U (1999) Rett syndrome and beyond: recurrent spontaneous and familial MECP2 mutations at CpG hotspots. *Am J Hum Genet* 65:1520–1529
- Webb T, Clarke A, Hanefeld F, Pereira JL, Rosenbloom L, Woods CG (1998) Linkage analysis in Rett syndrome families suggests that there may be a critical region at Xq28. *J Med Genet* 35:997–1003
- Whitty CJ, Kapatos G, Bannon MJ (1993) Neurotrophic effects of substance P on hippocampal neurons in vitro. *Neurosci Lett* 164:141–144
- Xiang F, Buervenich S, Nicolao P, Bailey ME, Zhang Z, Anvret M (2000) Mutation screening in Rett syndrome patients. *J Med Genet* 37:250–255
- Xiang F, Zhang Z, Clarke A, Jose Luiz P, Sakkubai N, Sarojini B, Delozier-Blanchet CD, Hansmann I, Edstrom L, Anvret M (1998) Chromosome mapping of Rett syndrome: a likely candidate region on the telomere of Xq. *J Med Genet* 35:297–300
- Yamada Y, Miura K, Kumagai T, Hayakawa C, Miyazaki S, Matsumoto A, Kurosawa K, Nomura N, Taniguchi H, Sonta SI, Yamanaka T, Wakamatsu N (2001) Molecular analysis of Japanese patients with Rett syndrome: identification of five novel mutations and genotype-phenotype correlation. *Hum Mutat* 18:253
- Yamashita Y, Kondo I, Fukuda T, Morishima R, Kusaga A, Iwanaga R, Matsuishi T (2001) Mutation analysis of the methyl-CpG-binding protein 2 gene (MECP2) in Rett patients with preserved speech. *Brain Dev* 23 Suppl 1: S157–S160
- Yoder JA, Walsh CP, Bestor TH (1997) Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* 13:335–340
- Yu F, Thiesen J, Stratling WH (2000) Histone deacetylase-independent transcriptional repression by methyl-CpG-binding protein 2. *Nucleic Acids Res* 28:2201–2206
- Yu F, Zingler N, Schumann G, Stratling WH (2001) Methyl-CpG-binding protein 2 represses LINE-1 expression and retrotransposition but not Alu transcription. *Nucleic Acids Res* 29:4493–4501
- Yusufzai TM, Wolffe AP (2000) Functional consequences of Rett syndrome mutations on human MeCP2. *Nucleic Acids Res* 28:4172–4179
- Zappella M (1992) The Rett girls with preserved speech. *Brain Dev* 14:98–101
- Zappella M, Meloni I, Longo I, Hayek G, Renieri A (2001) Preserved speech variants of the Rett syndrome: molecular and clinical analysis. *Am J Med Genet* 104:14–22
- Zeev BB, Yaron Y, Schanen NC, Wolf H, Brandt N, Ginot N, Shomrat R, Orr-Urtreger A (2002) Rett syndrome: clinical manifestations in males with MECP2 mutations. *J Child Neurol* 17:20–24
- Zoghbi H (1988) Genetic aspects of Rett syndrome. *J Child Neurol* 3:S76–S78
- Zoghbi HY, Percy AK, Schultz RJ, Fill C (1990) Patterns of X chromosome inactivation in the Rett syndrome. *Brain Dev* 12:131–135